

REMARKS

The Present Invention

The present invention pertains to isolated cancer peptides consisting of (a) about 10 contiguous amino acids of SEQ ID NO: 4 that include amino acids 55-62 of SEQ ID NO: 4 or amino acids 127-136 of SEQ ID NO: 4 and (b) optionally 1 to about 10 additional contiguous amino acids of SEQ ID NO: 4 at the N-terminus of the cancer peptide, functionally equivalent variants thereof, as well as compositions and immunogens, both of which comprise the cancer peptides.

The Pending Claims

Claims 3, 5-8, 10, 12-15, 26, 28, 29, 67-77, 83-85, and 87-103 are currently pending. All claims stand rejected under new grounds. Claims 3, 5-8, 10, 12-15, 26, 67-77, and 87 stand rejected under 35 U.S.C. 112, Second Paragraph, as allegedly being indefinite, and under 35 U.S.C. 112, First Paragraph, as allegedly failing to meet the written description and enablement requirements. In addition, claims 28-29 and 83-85 stand rejected under 35 U.S.C. 102(a). Finally, claim 87 is objected to under 37 CFR 1.45(c).

The Amendments to the Claims

Claims 26 and 28 have been amended to make them clearer. Claim 26 now incorporates the limitations of claim 3. Claim 28 has been amended to recite "comprising the composition of claim 26." No new matter has been added by way of these amendments.

Discussion of Claim Objection

Claim 87 stands objected to as being an improper dependent claim under 37 CFR 1.45(c). Specifically, the Office alleges claim 3 recites "[a]n isolated cancer peptide consisting of about 10 contiguous amino acids" and that claim 87 recites "wherein the cancer peptide is about 10 amino acids in length." Applicants respectfully traverse. Claim 3 encompasses peptides of about 20 amino acids, while 87 does not encompass a peptide with more than about 10 amino acids. Thus, Applicants request withdrawal of the objection.

Discussion of Claim Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 3, 5-8, 10, 12-15, 26, 67-77, and 87 stand rejected under 35 U.S.C. 112, Second Paragraph, as allegedly being indefinite. Specifically, the Office alleges that claims 3, 5-8, 10, 12-15, 26, 67-77, and 87 are indefinite for reciting "optionally" in claim 3. The MPEP specifically allows the use of "optionally" in claims (MPEP § 2173.05(h)(II)). The Office Action

also has not explained why the use of “optionally” in the rejected claims is confusing to the skilled artisan as is required (MPEP § 2173.05(h)(III)).

Claims 3, 5-8, 10, 12-15, 26, 67-77, and 87 also stand rejected as indefinite for reciting “functionally equivalent variants” in claim 3. The Office alleges that the phrase “functionally equivalent variants” encompasses peptides having disparate functions and that the specification does not provide a *standard* for ascertaining the direction, requisite degree or endpoint, of the changes to the cancer peptides. Therefore, the Office concludes that the skilled artisan would not reasonably be apprised of the metes and bounds of the invention. Applicants respectfully traverse. The ordinarily skilled artisan understands whether peptides are “immunologically recognized by T-cells.” Moreover, the specification provides routine assays in Examples 2, 3, 11-13, and 15 that provide *standards* for determining functionally equivalent variants. When read in light of these disclosures, claims 3, 5-8, 10, 12-15, 26, 67-77, and 87 appraise the skilled artisan of the meets and bounds of the invention.

For the forgoing reasons, Applicants request withdrawal of the Section 112, Second Paragraph, rejections.

Discussion of Claim Rejections under 35 U.S.C. § 112, First Paragraph, Written Description

Claims 3, 5-8, 10, 12-15, 26, 67-77, and 87-103 stand rejected under 35 U.S.C. 112, First Paragraph, for alleged lack of compliance with the written description requirement. First, noting that the claims are to functionally equivalent variants of SEQ ID NO: 4 that include amino acids 55-62 (which is SEQ ID NO: 31) or 127-136 (which is SEQ ID NO: 15), the Office alleges that the specification does not disclose any homologous cancer peptides. Alleging that such homologous cancer peptides can be broadly interpreted as being naturally occurring alleles the Office concludes that general knowledge in the art concerning such variants does not provide any indication of how the structure of one variant is representative of unknown variants. Furthermore, the Office alleges that the specification does not describe allelic variants or the structural or functional aspects of the claimed peptides. Thus, the Office Action also concludes, one of skill in the art cannot envision the detailed structure of the encompassed polypeptides. The Office further alleges that the amendments to claims 3, 5-8, 10, 12-15, 26, 67-77, and 87-103 filed on April 8, 2004 have introduced new matter. Specifically, the Office alleges that the original specification does not disclose the “narrower” claim scope that is encompassed by the claims to peptides with amino acids 54-62 (SEQ ID NO: 14), 53-62 (SEQ ID NO: 25), 48-62 (SEQ ID NO: 26), 43-62 (SEQ ID NO: 43), and 127-136 (SEQ ID NO: 15). Applicants respectfully traverse.

The specification explicitly recites nucleic acids having the sequences of positions 55-62 and 127-136 of SEQ ID NO: 4 as SEQ ID NOS: 31 and 15, which are shown in Tables 6

and 7 (pages 45 and 50). Furthermore, the specification explicitly recites that portions and variants are within the scope of the present invention (page 9, line 21). Moreover, Tables 6 and 7 give more than ample guidance for selecting such cancer peptides. Parenthetically, it is noted that these cancer peptides can be selected without undue experimentation. In this regard, Applicants note that there is no *per se* requirement to disclose a complete DNA sequence when claiming DNA sequences (See, e.g., the Office's Guidelines on the Written Description, Fed. Reg., Vol. 66, No. 4, p.1101, comment No. 9 (Jan. 5, 2001))(copy attached).

Furthermore, although operative examples are not required (MPEP § 2164.02), several operative claimed peptides are disclosed (*See, e.g.*, Tables 6 and 7.) In addition, structural requirements for function are disclosed at specification page 47, lines 4-19, and page 53, lines 4-15. Additionally, the applicant is not claiming *alleles*; the applicant is claiming isolated cancer peptides and functionally equivalent variants thereof, no matter where they are encoded in the genome. Because alleles are not claimed *per se*, an analysis of any alleged alleles is irrelevant.

The Office Action also supports the Section 112, First Paragraph rejection, by alleging that the disclosure at page 9 appears to only have contemplated variants having at least 85% sequence homology relative to the full-length cancer peptide of SEQ ID NO: 4 (i.e., cancer peptide comprising SEQ ID NO: 4) and not 85% sequence homology to the claimed peptides. But, the specification on page 9, at line 21, states “[a]lso encompassed in the ambit of the invention are cancer peptides *or portions thereof that share partial sequence homology with SEQ ID NO: 4*. By partial amino acid sequence homology is meant a peptide having at least 85% sequence homology with SEQ ID NO: 4...” Thus, the disclosure on page 9 encompasses portions of SEQ ID NO: 4 and those peptides with 85% homology to the portion of SEQ ID NO: 4 to which they correspond.

The Office Action further alleges that the data presented in Table 7 would not lead the skilled artisan to select a peptide with 85% homology to the SEQ ID NO: 4 sequence over any of the other peptides in Table 7 and that applicant has not pointed to where there is support for such a cancer peptide in the specification. In addition, it is alleged that adequate support for the cancer peptides consisting of amino acids 54-62 (SEQ ID NO: 33), 43-62, 48-62, 55-62 (claim 88) and 54-62 (claim 88) of SEQ ID NO: 4 cannot be found in the specification as filed.

The specification on page 9, at line 21, states “[a]lso encompassed in the ambit of the invention are cancer peptides *or portions thereof that share partial sequence homology with SEQ ID NO: 4*. By partial amino acid sequence homology is meant a peptide having at least 85% sequence homology with SEQ ID NO: 4...” Thus, the disclosure on page 9 includes portions of

SEQ ID NO: 4 and those peptides having 85% homology to their corresponding portions of SEQ ID NO: 4. Table 7 discloses sixteen peptides, including some with sequences longer than 10 amino acids, that maintain significant activity (e.g., SEQ ID NOS: 26-30). In addition, the specification discloses that longer peptides may be processed to shorter peptides on page 53, lines 4-15. Thus, the specification supports the claimed peptides made up from amino acids 54-62 (SEQ ID NO: 33), 43-62, 48-62, 55-62 (claim 88) and 54-62 (claim 88) of SEQ ID NO: 4.

The Office Action alleges that the rejected claims are drawn to contiguous additional amino acids at the N-terminus, whereas the specification appears to only disclose that any additional amino acids at the N-terminus. Applicants note that the specification states at page 9, line 21 states “[a]lso encompassed in the ambit of the invention are cancer peptides *or portions thereof* that share partial sequence homology with SEQ ID NO: 4.” Such portions would have to be limited by what a skilled artisan would know to be a reasonable length. As “*portions thereof*” such sequences can be contiguous amino acids, rather than several isolated, individual amino acids from SEQ ID NO: 4.

Finally, the Office alleges that Applicants’ reliance on a generic disclosure and a limited number of species does not provide sufficient direction and guidance to the currently claimed limitations. The Office further alleges that it cannot be said that a subgenus is necessarily described by a genus encompassing it and a species upon which it reads. Tables 6 and 7 provide nineteen distinct operative embodiments of the invention. The specification, at page 47, lines 4-19, and page 53, lines 4-15; teaches structural elements (amino acids) necessary for activity. Thus, the specification contains more than mere generic disclosure and the written description, as filed, supports the claims 3, 5-8, 10, 12-15, 26, 67-77, and 87-103.

Moreover, the aforementioned disclosures are explicit in the specification and there is no need for Applicants to rely on matter that is “obvious” from their disclosure but not disclosed. Therefore, Applicants request withdrawal of the written description rejection.

Discussion of Claim Rejections under 35 U.S.C. § 112, First Paragraph, Enablement

Claims 3, 5-8, 10, 12-15, 26, 28-29, 67-77, 83-85, and 87-103 stand rejected under 35 U.S.C. 112, First Paragraph, as failing to enable the invention. The Office alleges that specification provides insufficient guidance and provides no working examples of a cancer peptide of the instant invention that would work with any MHC molecule other than HLA-A31. In addition, the Office alleges that considering the state of art, the broad scope of claims, and the nature of MHC molecules there is insufficient guidance with respect to the broadly claimed variants and cancer peptides longer than 10 amino acids. Thus, the Office concludes that that undue experimentation would be required to practice the claimed invention.

More specifically, the Office alleges that the specification only teaches certain claimed species of SEQ ID NO: 4 in combination with HLA-A31 and not any other MHC class I molecule that can be recognized by Cytotoxic T Cells ("CTLs"). Therefore, the office alleges, it is not clear that reactive CTLs could be generated using the claimed cancer peptides in combination with HLA-A3, HLA-A11, HLA-A33 and HLA-A68 presentation. Furthermore, the Office cites Riott et al. (Immunology, Fourth Edition, 1996, Mosby, page 7.9-7.11) alleging that different MHC molecules bind different sets of peptides. Applicants respectfully traverse.

The skilled artisan would be aware of the reference of Falk et al. (Immunogenetics 40:238-241 (1994), disclosed in Applicant's IDS of 8/1/2000) that teaches HLA-A1, -A11, -A31, and -A33 molecules bind similar peptides. In addition, Missale et al. (J. Exp. Med. 177: 751-762 (1993)) teaches that both HLA-A31 and HLA-Aw68 CTLs respond to a single Hepatitis B epitope. The Falk and Missale references are more directly relevant to the claims at issue than the Riott reference. Thus, the skilled artisan would reasonably expect that cancer peptides recognized by HLA-A31 CTLs would also be recognized by HLA-A3, HLA-A11, HLA-A33 and HLA-A68 CTLs.

Similarly, the Office further alleges that specification does not teach just any functional equivalent variant cancer peptide, including some of the claimed cancer peptides. This allegation coupled with the alleged teachings Riott et al. leads the Office to conclude that the species encompassed by claim 3 would not work. The Office alleges that this conclusion is supported by the lack of activity found with some of the peptides disclosed in Tables 6 and 7. Applicants respectfully traverse.

That some inoperative embodiments may be encompassed by scope of a claim does not necessarily make the claim nonenabled. In such a setting the standard for nonenablement is whether a skilled artisan could determine which embodiments that are conceived, but not yet made, would be inoperative or operative without undue experimentation (MPEP § 2164.08(b)). The specification teaches on page 47, lines 4-19, and page 53, lines 4-15, structural elements (amino acids) necessary for activity. In addition, the specification, in Examples 10 and 11, gives extensive guidance on the performance of the routine experiments required to determine whether just any variant peptide is functionally equivalent to the working embodiments disclosed. Moreover, Tables 6 and 7 disclose that 19 of 41 (41%) of the peptides tested stimulate CTL activity indicating that there is a reasonable expectation that the skilled artisan would quickly distinguish operative from inoperative embodiments.

Next, the Office cites Wang et al. (U.S. Patent 5,840,839) and Bixler et al. (U.S. Patent 5,785,973) as suggesting that theoretically selected T cell binding motifs have to be tested experimentally in order to determine whether they are actually T cell epitopes or not.

Additionally, the Office cites Geysen (U.S. Patent 5,539,084) which allegedly teaches that even for peptides of similar size derived from the same "parent" polypeptide, not all will be capable of interacting with T-cells. Thus, the Office concludes that neither the specification, nor the prior, art teach the full-scope of cancer peptides claimed. Applicant respectfully traverses.

Wang et al.'s (U.S. Patent 5,840,839) circumstances are not analogous to those of the present application. Using a different amino acid sequence, Wang et al. failed to identify a peptide that significantly stimulated CTLs, while Applicant discloses nineteen peptides that *do* stimulate CTLs (Tables 6 and 7). Thus, the testing taught by Wang et al. with the present invention does not constitute undue experimentation. Bixler et al. (U.S. patent 5,785,973) teaches that "there is agreement among several groups using a variety of models that a region of 7-17 amino acid residues is required for [T-cell] recognition" (col. 6, line 21-26) in accordance with the instant claims. Finally, Geysen (U.S. Patent 5,539,084), teaches that not all peptides of similar size derived from the same "parent" polypeptide will interact with T-cells. This is not disputed by the Applicants. Applicants have had some similar results (Tables 6 and 7), but, the specification provides both extensive guidance and numerous active peptides in Examples 11 and 12. Thus, the patents cited by the Office do not indicate undue experimentation will be required to practice the instant invention.

Finally, the Office alleges the specification does not disclose common structural attributes that identify the claimed cancer peptides or functionally equivalent variants thereof. Therefore, the Office Action alleges that there is insufficient guidance regarding selection of peptides that meet the instant criteria of stimulating reactive T lymphocytes. Applicants respectfully traverse. Applicants' specification at page 47, lines 4-19, and page 53, lines 4-15, teach structural elements (amino acids) providing activity. Furthermore, the skilled artisan could use the guidance of Falk et al. (Immunogenetics 40:238-241 (1994)) and Missale et al. (J. Exp. Med. 177: 751-762 (1993)), which teach a similar peptide binding affinity for the HLA-A1, -A11, -A31, -A33, and -A68, when choosing CTLs.

For the above reasons, Applicants respectfully request withdrawal of the enablement rejection.

Discussion of Claim Rejection under U.S.C. § 102

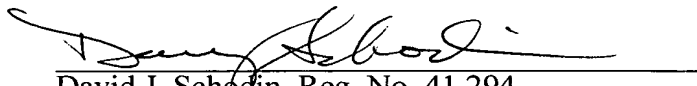
Claims 28-29 and 83-85 stand rejected under 35 U.S.C. 102(a) as being anticipated by Chen et al. (Proc. Natl. Acad. Sci. USA, 94:1914-1918 (1997)). The Office Action alleges that Chen et al. teaches an immunogenic antigen that comprises of about 10 contiguous amino acids of SEQ ID NO: 4. Applicants have amended claims 26 and 28 to make it more clear that the disclosure of Chen et al. is not encompassed by the subject of the claims. In view of these clarifying amendments of claims 26 and 28, the rejection should be withdrawn.

In re Appln. of Wang et al.
Application No. 09/529,206

Conclusion

The application is considered to be in good and proper form for allowance, and the Examiner is respectfully requested to pass this application to issue. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,



David J. Schodin, Reg. No. 41,294
LEYDIG, VOIT & MAYER, LTD.
Two Prudential Plaza, Suite 4900
180 North Stetson
Chicago, Illinois 60601-6780
(312) 616-5600 (telephone)
(312) 616-5700 (facsimile)

Date: September 22, 2004

an asserted utility, unless countervailing evidence can be provided that shows that one of ordinary skill in the art would have a legitimate basis to doubt the credibility of such a statement. Similarly, Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered.

Once a *prima facie* showing of no specific and substantial credible utility has been properly established, the applicant bears the burden of rebutting it. The applicant can do this by amending the claims, by providing reasoning or arguments, or by providing evidence in the form of a declaration under 37 CFR 1.132 or a patent or a printed publication that rebuts the basis or logic of the *prima facie* showing. If the applicant responds to the *prima facie* rejection, the Office personnel should review the original disclosure, any evidence relied upon in establishing the *prima facie* showing, any claim amendments, and any new reasoning or evidence provided by the applicant in support of an asserted specific and substantial credible utility. It is essential for Office personnel to recognize, fully consider and respond to each substantive element of any response to a rejection based on lack of utility. Only where the totality of the record continues to show that the asserted utility is not specific, substantial, and credible should a rejection based on lack of utility be maintained.

If the applicant satisfactorily rebuts a *prima facie* rejection based on lack of utility under § 101, withdraw the § 101 rejection and the corresponding rejection imposed under § 112, first paragraph.

Dated: December 29, 2000.

Q. Todd Dickinson,

Under Secretary of Commerce for Intellectual Property and Director of the United States Patent and Trademark Office.

[FR Doc. 01-322 Filed 1-4-01; 8:45 am]

BILLING CODE 3510-16-U

DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

[Docket No. 991027288-0264-02]

RIN 0651-AB10

Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1, "Written Description" Requirement

AGENCY: United States Patent and Trademark Office, Commerce.

ACTION: Notice.

SUMMARY: These Guidelines will be used by USPTO personnel in their review of patent applications for compliance with the "written description" requirement of 35 U.S.C. 112, ¶ 1. These Guidelines supersede the "Revised Interim Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 'Written Description' Requirement" that were published in the *Federal Register* at 64 FR 71427, Dec. 21, 1999, and in the *Official Gazette* at 1231 O.G. 123, Feb. 29, 2000. These Guidelines reflect the current understanding of the USPTO regarding the written description requirement of 35 U.S.C. 112, ¶ 1, and are applicable to all technologies.

DATES: The Guidelines are effective as of January 5, 2001.

FOR FURTHER INFORMATION CONTACT: Stephen Walsh by telephone at (703) 305-9035, by facsimile at (703) 305-9373, by mail to his attention addressed to United States Patent and Trademark Office, Box 8, Washington, DC 20231, or by electronic mail at "stephen.walsh@uspto.gov"; or Linda Therhorn by telephone at (703) 305-8800, by facsimile at (703) 305-8825, by mail addressed to Box Comments, Commissioner for Patents, Washington, DC 20231, or by electronic mail at "linda.therhorn@uspto.gov."

SUPPLEMENTARY INFORMATION: As of the publication date of this notice, these Guidelines will be used by USPTO personnel in their review of patent applications for compliance with the "written description" requirement of 35 U.S.C. 112, ¶ 1. Because these Guidelines only govern internal practices, they are exempt from notice and comment rulemaking under 5 U.S.C. 553(b)(A).

Discussion of Public Comments

Comments were received from 48 individuals and 18 organizations in response to the request for comments on the "Revised Interim Guidelines for Examination of Patent Applications

Under the 35 U.S.C. 112, ¶ 1 'Written Description' Requirement" published in the *Federal Register* at 64 FR 71427, Dec. 21, 1999, and in the *Official Gazette* at 1231 O.G. 123, Feb. 29, 2000. The written comments have been carefully considered.

Overview of Comments

The majority of comments favored issuance of final written description guidelines with minor revisions. Comments pertaining to the written description guidelines are addressed in detail below. A few comments addressed particular concerns with respect to the associated examiner training materials that are available for public inspection at the USPTO web site (www.uspto.gov). Such comments will be taken under advisement in the revision of the training materials; consequently, these comments are not specifically addressed below as they do not impact the content of the Guidelines. Several comments raised issues pertaining to the patentability of ESTs, genes, or genomic inventions with respect to subject matter eligibility (35 U.S.C. 101), novelty (35 U.S.C. 102), or obviousness (35 U.S.C. 103). As these comments do not pertain to the written description requirement under 35 U.S.C. 112, they have not been addressed. However, the aforementioned comments are fully addressed in the "Discussion of Public Comments" in the "Utility Examination Guidelines" Final Notice, which will be published at or about the same time as the present Guidelines.

Responses to Specific Comments

(1) *Comment:* One comment stated that the Guidelines instruct the patent examiner to determine the correspondence between what applicant has described as the essential identifying characteristic features of the invention and what applicant has claimed, and that such analysis will lead to error. According to the comment, the examiner may decide what applicant should have claimed and reject the claim for failure to claim what the examiner considers to be the invention. Another comment suggested that the Guidelines should clarify what is meant by "essential features of the invention." Another comment suggested that what applicant has identified as the "essential distinguishing characteristics" of the invention should be understood in terms of *Fiers v. Revel*, 984 F.2d 1164, 1169, 25 USPQ2d 1601, 1605 (Fed. Cir. 1993) ("Conception of a substance claimed *per se* without reference to a process requires conception of its structure, name,

BEST AVAILABLE COPY

formula, or definitive chemical or physical properties.”).

Response: The suggestions have been adopted in part. The purpose of the written description analysis is to confirm that applicant had possession of what is claimed. The Guidelines have been modified to instruct the examiners to compare the scope of the invention claimed with the scope of what applicant has defined in the description of the invention. That is, the Guidelines instruct the examiner to look for consistency between a claim and what provides adequate factual support for the claim as judged by one of ordinary skill in the art from reading the corresponding written description.

(2) *Comment:* Two comments urge that *Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 43 USPQ2d 1398 (Fed. Cir. 1997), is bad law and should not be followed by the USPTO because it conflicts with binding precedent, such as *Vas-Cath v. Mahurkar*, 935 F.2d 1555, 19 USPQ2d 1111 (Fed. Cir. 1991). *Response:* The final Guidelines are based on the Office's current understanding of the law and are believed to be fully consistent with binding precedent of the U.S. Supreme Court and the U.S. Court of Appeals for the Federal Circuit. *Eli Lilly* is a precedential decision by the Court that has exclusive jurisdiction over appeals involving patent law. Accordingly, the USPTO must follow *Eli Lilly*. Furthermore, the USPTO does not view *Eli Lilly* as conflicting with *Vas-Cath*. *Vas-Cath* explains that the purpose of the written description requirement is to ensure that the applicant has conveyed to those of skill in the art that he or she was in possession of the claimed invention at the time of filing. *Vas-Cath*, 935 F.2d at 1563–64, 19 USPQ2d at 1117. *Eli Lilly* explains that a chemical compound's name does not necessarily convey a written description of the named chemical compound, particularly when a genus of compounds is claimed. *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1405. The name, if it does no more than distinguish the claimed genus from all others by function, does not satisfy the written description requirement because “it does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus.” *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406. Thus, *Eli Lilly* identified a set of circumstances in which the words of the claim did not, without more, adequately convey to

others that applicants had possession of what they claimed.

(3) *Comment:* Several comments urged that the Guidelines do not recognize the inconsistency between the original claim doctrine and the written description requirement as set out in *Fiers* and *Eli Lilly*. On the other hand, another comment asserts that there is no strong presumption that an originally filed claim constitutes an adequate written description of the claimed subject matter. Several comments indicate that *in haec verba* support should be sufficient to comply with the written description requirement. Two comments urge that the concept of constructive reduction to practice upon filing of an application has been ignored. *Response:* As noted above, the USPTO does not find *Fiers* and *Eli Lilly* to be in conflict with binding precedent. An original claim may provide written description for itself, but it still must be an adequate written description which establishes that the inventor was in possession of the invention. The “original claim doctrine” is founded on cases which stand for the proposition that originally filed claims are part of the written description of an application as filed, and thus subject matter which is present only in originally filed claims need not find independent support in the specification. See, e.g., *In re Koller*, 613 F.2d 819, 824, 204 USPQ 702, 706 (CCPA 1980) (later added claims of similar scope and wording were adequately described by original claims); *In re Gardner*, 480 F.2d 879, 880, 178 USPQ 149, 149 (CCPA 1973) (“Under these circumstances, we consider the original claim in itself adequate ‘written description’ of the claimed invention. It was equally a ‘written description’ * * * whether located among the original claims or in the descriptive part of the specification.”). However, as noted in the preceding comment, *Eli Lilly* identified a set of circumstances in which the words of the claim did not, without more, adequately convey to others that applicants had possession of what they claimed. When the name of a novel chemical compound does not convey sufficient structural information about the compound to identify the compound, merely reciting the name is not enough to show that the inventor had possession of the compound at the time the name was written. The Guidelines indicate that there is a “strong presumption” that an adequate written description of the claimed invention is present when the application is filed, consistent with *In re Wertheim*, 541 F.2d 257, 263, 191 USPQ

90, 97 (CCPA 1976) (“we are of the opinion that the PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims.”). In most cases, the statement that “an originally filed claim is its own written description,” is borne out because the claim language conveys to others of skill in the art that the applicant was “in possession” of what is claimed. The Guidelines emphasize that the burden of proof is on the examiner to establish that a description as filed is not adequate and require the examiner to introduce sufficient evidence or technical reasoning to shift the burden of going forward with contrary evidence to the applicant.

(4) *Comment:* One comment stated that the Guidelines change the substance of the written description requirement to require some level of enablement. The comment stated that the *Eli Lilly* case should not be followed because its change in the quality of the description required is in conflict with precedent. Another comment suggested that to comply with the written description requirement, the description must both (i) demonstrate possession of the claimed invention by the applicant; and (ii) put the public in possession of the claimed invention. *Response:* As noted in the comment above, the USPTO is bound by the Federal Circuit's decision in *Eli Lilly*. The Guidelines have been revised to clarify that an applicant must provide a description of the claimed invention which shows that applicant was in possession of the claimed invention. The suggestion to emphasize that the written description requirement must put the public in possession of the invention has not been adopted because it removes much of the distinction between the written description requirement and the enablement requirement. Although the two concepts are entwined, they are distinct and each is evaluated under separate legal criteria. The written description requirement, a question of fact, ensures that the inventor conveys to others that he or she had possession of the claimed invention; whereas, the enablement requirement, a question of law, ensures that the inventor conveys to others how to make and use the claimed invention.

(5) *Comment:* One comment suggested that the Guidelines should provide examples of situations in which the written description requirement was met but the enablement requirement was not, and vice versa. Another comment stated that examiners often use enablement language in making

written description rejections.

Response: The enablement and written description requirements are not coextensive and, therefore, situations will arise in which one requirement is met but the other is not. Federal Circuit case law demonstrates many circumstances where enablement or written description issues, but not both, were before the Court. These Guidelines are intended to clarify for the examining corps the criteria needed to satisfy the written description requirement. For examples applying these Guidelines to hypothetical fact situations, see the "Synopsis of Application of Written Description Guidelines" (examiner training materials available on-line at <http://www.uspto.gov/web/menu/written.pdf>). These examples, as well as the examination form paragraphs and instructions on their proper use, provide the appropriate language examiners should use in making written description rejections.

(6) **Comment:** One comment disagreed with the statement in an endnote that "the fact that a great deal more than just a process is necessary to render a product invention obvious means that a great deal more than just a process is necessary to provide written description for a product invention." The comment indicated that the statement is overly broad and inconsistent with the "strong presumption that an adequate written description of the claimed invention is present when the application is filed." As an extreme case, for example, for product-by-process claims, nothing else would be needed to provide the written description of the product. **Response:** The endnote has been clarified and is now more narrowly drawn. However, there is no *per se* rule that disclosure of a process is sufficient to adequately describe the products produced by the process. In fact, *Fiers v. Revel* and *Eli Lilly* involved special circumstances where the disclosure of a process of making and the function of the product alone did not provide an adequate written description for product claims. Even when a product is claimed in a product-by-process format, the adequacy of the written description of the process to support product claims must be evaluated on a case-by-case basis.

(7) **Comment:** Several comments urge that actual reduction to practice, as a method of satisfying the written description requirement by demonstrating possession, has been over-emphasized. **Response:** The Guidelines have been clarified to state that describing an actual reduction to practice is one of a number of ways to show possession of the invention.

Description of an actual reduction to practice offers an important "safe haven" that applies to all applications and is just one of several ways by which an applicant may demonstrate possession of the claimed invention. Actual reduction to practice may be crucial in the relatively rare instances where the level of knowledge and level of skill are such that those of skill in the art cannot describe a composition structurally, or specify a process of making a composition by naming components and combining steps, in such a way as to distinguish the composition with particularity from all others. Thus, the emphasis on actual reduction to practice is appropriate in those cases where the inventor cannot provide an adequate description of what the composition is, and a definition by function is insufficient to define a composition "because it is only an indication of what the [composition] does, rather than what it is." *Eli Lilly*, 119 F.3d at 1568, 43 USPQ at 1406. See also *Amgen Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991).

(8) **Comment:** One comment asserts that the citation to *Pfaff v. Wells Electronics, Inc.*, 525 U.S. 55, 48 USPQ2d 1641 (1998) is inappropriate and should be deleted because *Pfaff* is concerned with § 102(b) on-sale bar, not written description. Another comment suggested that the Guidelines should provide an explanation of how the "ready for patenting" concept of *Pfaff* should be used in determining compliance with the written description requirement. **Response:** The Guidelines state the general principle that actual reduction to practice is not required to show possession of, or to adequately describe, a claimed invention (although, as noted in the previous comment, an actual reduction to practice is crucial in relatively rare instances). An alternative is to show that the invention described was "ready for patenting" as set out in *Pfaff*. For example, a description of activities that demonstrates the invention was "ready for patenting" satisfies the written description requirement. As *Wertheim* indicates, "how the specification accomplishes this is not material." 541 F.2d at 262, 191 USPQ at 96.

(9) **Comment:** One comment stated that the written description of a claimed DNA should be required to include the complete sequence of the DNA and claims should be limited to the DNA sequence disclosed. **Response:** Describing the complete chemical structure, *i.e.*, the DNA sequence, of a claimed DNA is one method of

satisfying the written description requirement, but it is not the only method. See *Eli Lilly*, 119 F.3d at 1566, 43 USPQ2d at 1404 ("An adequate written description of a DNA * * * requires a precise definition, *such as* by structure, formula, chemical name, or physical properties." (emphasis added, internal quote omitted)). Therefore, there is no basis for a *per se* rule requiring disclosure of complete DNA sequences or limiting DNA claims to only the sequence disclosed.

(10) **Comment:** One comment stated that it is difficult to envision how one could provide a description of sufficient identifying characteristics of the invention without physical possession of a species of the invention, and thus this manner of showing possession should be considered as a way to show actual reduction to practice. **Response:** This suggestion has not been adopted. The three ways of demonstrating possession as set forth in the Guidelines are merely exemplary and are not mutually exclusive. While there are some cases where a description of sufficient relevant identifying characteristics will evidence an actual reduction to practice, there are other cases where it will not. See, *e.g.*, *Ralston Purina Co. v. Far-Mar-Co, Inc.*, 772 F.2d 1570, 1576, 227 USPQ 177, 180 (Fed. Cir. 1985) (disclosure taken with the knowledge of those skilled in the art may be sufficient support for claims).

(11) **Comment:** One comment stated that the Guidelines should be revised to indicate that the test of disclosure of sufficiently detailed drawings should be expanded to include structural claiming of chemical entities. **Response:** The suggestion has been adopted.

(12) **Comment:** One comment stated that the Guidelines should reflect that an inventor is in possession of the invention when the inventor demonstrably has at least a complete conception thereof, and that factors and attributes which provide proof of written description should include evidence typically provided to prove a complete conception. **Response:** The suggestion has not been adopted because the conception analysis typically involves documentary evidence in addition to the description of the invention in the application as filed. However, it is acknowledged that if evidence typically provided to prove a complete conception is present in the specification as filed, it would be sufficient to show possession. The Federal Circuit has stated "[t]he conception analysis necessarily turns on the inventor's ability to describe his invention with particularity. Until he can do so, he cannot prove possession

of the complete mental picture of the invention." *Burroughs Wellcome Co. v. Barr Labs., Inc.*, 40 F.3d 1223, 1228, 32 USPQ2d 1915, 1919 (Fed. Cir. 1994). As further noted by the Federal Circuit, in order to prove conception, "a party must show possession of every feature recited in the count, and that every limitation of the count must have been known to the inventor at the time of the alleged conception." *Coleman v. Dines*, 754 F.2d 353, 359, 224 USPQ 857, 862 (Fed. Cir. 1985).

(13) *Comment*: One comment indicated that a "possession" test does not appear in Title 35 of the U.S. Code and is not clearly stated by the Federal Circuit. Therefore, it is recommended that patent examiners be directed to use existing judicial precedent to make rejections of claims unsupported by a statutory written description requirement. *Response*: While the Federal Circuit has not specifically laid out a "possession" test, the Court has clearly indicated that possession is a cornerstone of the written description inquiry. *See, e.g., Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991); *see also Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1323, 56 USPQ2d 1481, 1483 (Fed. Cir. 2000) ("[o]ne skilled in the art, reading the disclosure, must immediately discern the limitation at issue in the claims") (internal quote omitted). The possession test as set forth in the Guidelines is extrapolated from case law in a wide variety of technologies and is not intended to be limiting. Any rejections made by examiners will be made under 35 U.S.C. 112, ¶1, with supporting rationale. Final rejections are appealable if applicant disagrees and follows the required procedures to appeal.

(14) *Comment*: Two comments indicated that if the amino acid sequence for a polypeptide whose utility has been identified is described, then the question of possession of a class of nucleotides encoding that polypeptide can be addressed as a relatively routine matter using the understanding of the genetic code, and that the endnote addressing this issue should be revised. *Response*: The suggestion of these comments has been incorporated in the Guidelines and will be reflected in the training materials. However, based upon *In re Bell*, 991 F.2d 781, 785, 26 USPQ2d 1529, 1532 (Fed. Cir. 1993) and *In re Baird*, 16 F.3d 380, 382, 29 USPQ2d 1550, 1552 (Fed. Cir. 1994), this does not mean that applicant was in possession of any particular species of the broad genus.

(15) *Comment*: One comment disagreed with an endnote which stated

that a laundry list disclosure of moieties does not constitute a written description of every species in a genus. Specifically, the comment indicates that if the existence of a functional genus is adequately described in the specification, a laundry list of the species within that genus must satisfy the written description requirement.

Response: The suggestion to revise the endnote will not be adopted. A lack of adequate written description problem arises if the knowledge and level of skill in the art would not permit one skilled in the art to immediately envisage the product claimed from the disclosure. This was aptly demonstrated in *In re Bell* and *In re Baird* where possession of a large genus did not put a person of ordinary skill in the art in possession of any particular species. *See also Purdue Pharma*, 230 F.3d at 1328, 56 USPQ2d at 1487 (because the original specification did not disclose the later claimed concentration ratio was a part of the invention, the inventors cannot argue that they are merely narrowing a broad invention).

(16) *Comment*: One comment suggested that in the majority of cases, a single species will support a generic claim, and that the Guidelines should emphasize this point. *Response*: The suggestion has been adopted to a limited degree. The Guidelines now indicate that a single species may, in some instances, provide an adequate written description of a generic claim when the description of the species would evidence to one of ordinary skill in the art that the invention includes the genus. Note, however, *Tronzo v. Biomet, Inc.*, 156 F.3d 1154, 47 USPQ2d 1829 (Fed. Cir. 1998), where the species in the parent application was held not to provide written description support for the genus in the child application.

(17) *Comment*: One comment asserted that the Guidelines should focus on the compliance of the claims, not the specification, with the written description requirement. *Response*: This suggestion will not be adopted. "The specification shall contain a written description of the invention." 35 U.S.C. 112. The claims are part of the specification. *Id.*, ¶ 2. If an adequate description is provided, it will suffice "whether located among the original claims or in the descriptive part of the specification." *In re Gardner*, 480 F.2d 879, 880, 178 USPQ 149 (CCPA 1973). The entire disclosure, including the specification, drawings, and claims, must be considered.

(18) *Comment*: One comment asserted that the Guidelines confuse "new matter," 35 U.S.C. 132, with the written description requirement, and that the

same standard for written description should be applied to both original claims and new or amended claims.

Response: The Guidelines indicate that for both original and amended claims, the inquiry is whether one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention at the time the application was filed.

(19) *Comment*: One comment suggested that the second paragraph of the section pertaining to determining what the claim as a whole covers should be deleted because it relates more to compliance with § 112, second paragraph, than with the written description requirement. *Response*: This suggestion will not be adopted. The claims must be construed and all issues as to the scope and meaning of the claim must be explored during the inquiry into whether the written description requirement has been met. The concept of treating the claim as a whole is applicable to all criteria for patentability.

(20) *Comment*: One comment suggested a different order for the general analysis for determining compliance with the written description requirement, starting with reading the claim, then the specification, and then determining whether the disclosure demonstrates possession by the applicant. *Response*: This suggestion will not be adopted. The claims must be construed as broadly as reasonable in light of the specification and the knowledge in the art. *See In re Morris*, 127 F.3d 1048, 1054, 44 USPQ2d 1023, 1027 (Fed. Cir. 1997). Then the disclosure must be evaluated to determine whether it adequately describes the claimed invention, i.e., whether it conveys to a person having ordinary skill in the art that the applicant had possession of what he or she now claims.

(21) *Comment*: Several comments suggested that the Guidelines are unclear with regard to how the examiner should treat the transitional phrase "consisting essentially of." The comments also suggested that the endnote that explains "consisting essentially of" does not make clear how the use of this intermediate transitional language affects the scope of the claim. Several comments stated that the USPTO does not have legal authority to treat claims reciting this language as open (equivalent to "comprising"). Another comment suggested that the phrase "clear indication in the specification" be replaced with "explicit or implicit indication." *Response*: The transitional phrase "consisting essentially of" "excludes

ingredients that would 'materially affect the basic and novel characteristics' of the claimed composition." *Atlas Powder Co. v. E.I. DuPont de Nemours & Co.*, 750 F.2d 1569, 1574, 224 USPQ 409, 412 (Fed. Cir. 1984). The basic and novel characteristics of the claimed invention are limited by the balance of the claim. *In re Janakirama-Rao*, 317 F.2d 951, 954, 137 USPQ 893, 896 (CCPA 1963). However, during prosecution claims must be read broadly, consistent with the specification. *In re Morris*, 127 F.3d 1048, 1054, 44 USPQ2d 1023, 1027 (Fed. Cir. 1997). Thus, for purposes of searching for and applying prior art in a rejection under 35 U.S.C. 102 or 103, if the specification or the claims do not define the "basic and novel" properties of the claimed subject matter (or if such properties are in dispute), the broadest reasonable interpretation consistent with the specification is that the basic and novel characteristics are merely the presence of the recited limitations. See, e.g., *Janakirama-Rao*, 317 F.2d at 954, 137 USPQ at 895-96. This does not indicate that the intermediate transitional language is never given weight. Applicants may amend the claims to avoid the rejections or seek to establish that the specification provides definitions of terms in the claims that define the basic and novel characteristics of the claimed invention which distinguish the claimed invention from the prior art. When an applicant contends that additional steps or materials in the prior art are excluded by the recitation of 'consisting essentially of,' applicant has the burden of showing that the introduction of additional steps or components would materially change the characteristics of applicant's invention. *In re De Lajarte*, 337 F.2d 870, 143 USPQ 256 (CCPA 1964). The language used in the Guidelines is consistent with *PPG Industries Inc. v. Guardian Industries Corp.*, 156 F.3d 1351, 1355, 48 USPQ2d 1351, 1355 (Fed. Cir. 1998) ("PPG could have defined the scope of the phrase 'consisting essentially of' for purposes of its patent by making clear in its specification what it regarded as constituting a material change in the basic and novel characteristics.").

(22) *Comment*: One comment stated that the written description should "disclose the invention," including why the invention works and how it was developed. *Response*: This suggestion has not been adopted. An inventor does not need to know how or why the invention works in order to obtain a patent. *Newman v. Quigg*, 877 F.2d 1575, 1581, 11 USPQ2d 1340, 1345

(Fed. Cir. 1989). To satisfy the enablement requirement of 35 U.S.C. 112, ¶1, an application must disclose the claimed invention in sufficient detail to enable a person of ordinary skill in the art to make and use the claimed invention. To satisfy the written description requirement of 35 U.S.C. 112, ¶1, the description must show that the applicant was in possession of the claimed invention at the time of filing. There is no statutory basis to require disclosure of why an invention works or how it was developed. "Patentability shall not be negated by the manner in which the invention was made." 35 U.S.C. 103(a).

(23) *Comment*: One comment recommended that the phrases "emerging and unpredictable technologies" and "unpredictable art" be replaced with the phrase—inventions characterized by factors which are not reasonably predictable in terms of the ordinary skill in the art—. *Response*: The suggestion is adopted in part and the recommended phrase has been added as an alternative.

(24) *Comment*: One comment recommended that the phrase "conventional in the art" be replaced with—part of the knowledge of one of ordinary skill in the art—. *Response*: The suggestion is adopted in part and the recommended phrase has been added as an alternative. The standard of "conventional in the art" is supported by case law holding that a patent specification "need not teach, and preferably omits, what is well known in the art." See *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 1534, 3 USPQ2d 1737, 1743 (Fed. Cir. 1987); *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986). See also *Atmel Corp. v. Information Storage Devices, Inc.*, 198 F.3d 1374, 1382, 53 USPQ2d 1225, 1231 (Fed. Cir. 1999).

(25) *Comment*: One comment recommended that the Guidelines be amended to state that the appropriate skill level for determining possession of the claimed invention is that of a person of ordinary skill in the art. *Response*: The comment has not been adopted. The statutory language itself indicates that compliance with the requirements of 35 U.S.C. 112, ¶1, is judged from the standard of "any person skilled in the art." It is noted, however, that the phrases "one of skill in the art" and "one of ordinary skill in the art" appear to be synonymous. See, e.g., *Union Oil Co. v. Atlantic Richfield Co.*, 208 F.3d 989, 997, 54 USPQ2d 1227, 1232 (Fed. Cir. 2000) ("The written description requirement does not require the applicant to describe exactly the subject

matter claimed, [instead] the description must clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed." Thus, § 112, ¶ 1, ensures that, as of the filing date, the inventor conveyed with reasonable clarity to those of skill in the art that he was in possession of the subject matter of the claims." (citations omitted, emphasis added)).

(26) *Comment*: One comment stated that an endnote misstates the relevant law in stating that, to show inherent written descriptive support for a claim limitation, the inherent disclosure must be such as would be recognized by a person of ordinary skill in the art. The comment recommended that the endnote be amended to delete the reference to recognition by persons of ordinary skill and to cite *Pingree v. Hull*, 518 F.2d 624, 186 USPQ 248 (CCPA 1975), rather than *In re Robertson*, 169 F.3d 743, 49 USPQ2d 1949 (Fed. Cir. 1999). *Response*: The comment has not been adopted. Federal Circuit precedent makes clear that an inherent disclosure must be recognized by those of ordinary skill in the art. See, e.g., *Hyatt v. Boone*, 146 F.3d 1348, 1354-55, 47 USPQ2d 1128, 1132 (Fed. Cir. 1998) ("[T]he purpose of the description requirement is 'to ensure that the inventor had possession, as of the filing date of the application relied on, of the specific subject matter later claimed by him.' * * * Thus, the written description must include all of the limitations of the interference count, or the applicant must show that any absent text is necessarily comprehended in the description provided and would have been so understood at the time the patent application was filed." (emphasis added)). See also *Reiffin v. Microsoft Corp.*, 214 F.3d 1342, 1346, 54 USPQ2d 1915, 1917 (Fed. Cir. 2000) (The "application considered as a whole must convey to one of ordinary skill in the art, either explicitly or inherently, that [the inventor] invented the subject matter claimed * * *. See * * * *Continental Can Co. USA v. Monsanto Co.*, 948 F.2d 1264, 1268, 20 USPQ2d 1746, 1749 (Fed. Cir. 1991) (descriptive matter may be inherently present in a specification if one skilled in the art would necessarily recognize such a disclosure)").

(27) *Comment*: Several comments pointed out an inconsistency in the Federal Register Notice re: the Revised Interim Written Description Guidelines. The inconsistency concerned the treatment of claims directed to an isolated DNA comprising SEQ ID NO:1 wherein SEQ ID NO:1 is an expressed sequence tag. The comments contrasted paragraphs 34 and 35 of the Response to

Public Comments with the statement in the text of the Guidelines that a genus must be supported by a representative number of species (as analyzed in Example 7 of the training materials). *Response:* The USPTO acknowledges that there was an inconsistency. The Office notes that a claim reciting a nucleic acid comprising SEQ ID NO:1 may be subject to a rejection for lack of an adequate written description where particular identifiable species within the scope of the claim lack an adequate written description. The training materials as amended exemplify an appropriate analysis.

(28) *Comment:* One comment stated that the USPTO should respond to the issue of whether the U.S. is meeting its TRIPs obligations. This comment noted that the USPTO did not address an earlier comment regarding the "Interim Guidelines for the Examination of Patent Applications under the 35 U.S.C. 112, ¶ 1, 'Written Description' Requirement," 63 FR 32,639, June 15, 1998, which questioned whether the written description requirement is truly different from the enablement requirement, and indicated that such a requirement may be contrary to the TRIPs provisions of the World Trade Organization (Article 27.1). Article 27.1 requires WTO Members to, *inter alia*, make patents available, with limited exceptions, for products and processes in all fields of technology so long as those products and processes are new, involve an inventive step, and are capable of industrial application. The comment further suggested a response. *Response:* TRIPs Article 27 does not address what must be included in a patent application to allow WTO Member officials to determine whether particular inventions meet the standards for patentability established in that Article. TRIPs Article 29, which is more relevant to this comment, states that Members "shall require" patent applicants to disclose their invention "in a manner sufficiently clear and complete for the invention to be carried out by a person skilled in the art." If the written description is not clear and complete, the applicant may not have been in possession of the invention. This may support both written description and enablement standards. In addition, Article 29 expressly authorizes Members to require patent applicants to disclose the best method the inventor knows at the time of filing an application for carrying out the invention.

(29) *Comment:* Two comments commended the USPTO for eliminating the Biotechnology Specific Examples in the Revised Interim Written Description

Guidelines and providing separate training materials. One comment indicated a need to reconfirm the examples set forth in the Interim Written Description Guidelines published in 1998. *Response:* The current training materials reflect the manner in which the USPTO interprets the Written Description Guidelines.

(30) *Comment:* Several comments addressed specific concerns about the examiner training materials. *Response:* The comments received with respect to the training materials will be taken under advisement as the Office revises the training materials in view of the revisions to the Guidelines. The specific comments will not be addressed herein as they do not impact the language of the Guidelines.

Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1, "Written Description" Requirement

These "Written Description Guidelines" are intended to assist Office personnel in the examination of patent applications for compliance with the written description requirement of 35 U.S.C. 112, ¶ 1. This revision is based on the Office's current understanding of the law and public comments received in response to the USPTO's previous request for public comments on its Revised Interim Written Description Guidelines and is believed to be fully consistent with binding precedent of the U.S. Supreme Court, as well as the U.S. Court of Appeals for the Federal Circuit and its predecessor courts.

This revision does not constitute substantive rulemaking and hence does not have the force and effect of law. It is designed to assist Office personnel in analyzing claimed subject matter for compliance with substantive law. Rejections will be based upon the substantive law, and it is these rejections which are appealable. Consequently, any perceived failure by Office personnel to follow these Guidelines is neither appealable nor petitionable.

These Guidelines are intended to form part of the normal examination process. Thus, where Office personnel establish a *prima facie* case of lack of written description for a claim, a thorough review of the prior art and examination on the merits for compliance with the other statutory requirements, including those of 35 U.S.C. 101, 102, 103, and 112, is to be conducted prior to completing an Office action which includes a rejection for lack of written description. Office personnel are to rely on this revision of the Guidelines in the event of any inconsistent treatment of

issues involving the written description requirement between these Guidelines and any earlier guidance provided from the Office.

I. General Principles Governing Compliance With the "Written Description" Requirement for Applications

The first paragraph of 35 U.S.C. 112 requires that the "specification shall contain a written description of the invention * * *." This requirement is separate and distinct from the enablement requirement.¹ The written description requirement has several policy objectives. "[T]he 'essential goal' of the description of the invention requirement is to clearly convey the information that an applicant has invented the subject matter which is claimed."² Another objective is to put the public in possession of what the applicant claims as the invention.³ The written description requirement of the Patent Act promotes the progress of the useful arts by ensuring that patentees adequately describe their inventions in their patent specifications in exchange for the right to exclude others from practicing the invention for the duration of the patent's term.

To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention.⁴ An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention.⁵ Possession may be shown in a variety of ways including description of an actual reduction to practice,⁶ or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas that show that the invention was complete,⁷ or by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention.⁸ A question as to whether a specification provides an adequate written description may arise in the context of an original claim which is not described sufficiently, a new or amended claim wherein a claim limitation has been added or removed, or a claim to entitlement of an earlier priority date or effective filing date under 35 U.S.C. 119, 120, or 365(c).⁹ Compliance with the written description requirement is a question of

fact which must be resolved on a case-by-case basis.¹⁰

A. Original Claims

There is a strong presumption that an adequate written description of the claimed invention is present when the application is filed.¹¹ However, the issue of a lack of adequate written description may arise even for an original claim when an aspect of the claimed invention has not been described with sufficient particularity such that one skilled in the art would recognize that the applicant had possession of the claimed invention.¹² The claimed invention as a whole may not be adequately described if the claims require an essential or critical feature which is not adequately described in the specification and which is not conventional in the art or known to one of ordinary skill in the art.¹³ This problem may arise where an invention is described solely in terms of a method of its making coupled with its function and there is no described or art-recognized correlation or relationship between the structure of the invention and its function.¹⁴ A lack of adequate written description issue also arises if the knowledge and level of skill in the art would not permit one skilled in the art to immediately envisage the product claimed from the disclosed process.¹⁵

B. New or Amended Claims

The proscription against the introduction of new matter in a patent application¹⁶ serves to prevent an applicant from adding information that goes beyond the subject matter originally filed.¹⁷ Thus, the written description requirement prevents an applicant from claiming subject matter that was not adequately described in the specification as filed. New or amended claims which introduce elements or limitations which are not supported by the as-filed disclosure violate the written description requirement.¹⁸ While there is no *in haec verba* requirement, newly added claim limitations must be supported in the specification through express, implicit, or inherent disclosure. An amendment to correct an obvious error does not constitute new matter where one skilled in the art would not only recognize the existence of the error in the specification, but also recognize the appropriate correction.¹⁹ Deposits made after the application filing date cannot be relied upon to support additions to or correction of information in the application as filed.²⁰

Under certain circumstances, omission of a limitation can raise an

issue regarding whether the inventor had possession of a broader, more generic invention.²¹ A claim that omits an element which applicant describes as an essential or critical feature of the invention originally disclosed does not comply with the written description requirement.²²

The fundamental factual inquiry is whether the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, applicant was in possession of the invention as now claimed.²³

II. Methodology for Determining Adequacy of Written Description

A. Read and Analyze the Specification for Compliance With 35 U.S.C. 112, § 1

Office personnel should adhere to the following procedures when reviewing patent applications for compliance with the written description requirement of 35 U.S.C. 112, § 1. The examiner has the initial burden, after a thorough reading and evaluation of the content of the application, of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims. There is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed;²⁴ however, with respect to newly added or amended claims, applicant should show support in the original disclosure for the new or amended claims.²⁵ Consequently, rejection of an original claim for lack of written description should be rare. The inquiry into whether the description requirement is met is a question of fact that must be determined on a case-by-case basis.²⁶

1. For Each Claim, Determine What the Claim as a Whole Covers

Claim construction is an essential part of the examination process. Each claim must be separately analyzed and given its broadest reasonable interpretation in light of and consistent with the written description.²⁷ The entire claim must be considered, including the preamble language²⁸ and the transitional phrase.²⁹ The claim as a whole, including all limitations found in the preamble,³⁰ the transitional phrase, and the body of the claim, must be sufficiently supported to satisfy the written description requirement.³¹

The examiner should evaluate each claim to determine if sufficient structures, acts, or functions are recited to make clear the scope and meaning of the claim, including the weight to be given the preamble.³² The absence of definitions or details for well-

established terms or procedures should not be the basis of a rejection under 35 U.S.C. 112, § 1, for lack of adequate written description. Limitations may not, however, be imported into the claims from the specification.

2. Review the Entire Application to Understand How Applicant Provides Support for the Claimed Invention Including Each Element and/or Step

Prior to determining whether the disclosure satisfies the written description requirement for the claimed subject matter, the examiner should review the claims and the entire specification, including the specific embodiments, figures, and sequence listings, to understand how applicant provides support for the various features of the claimed invention.³³ The analysis of whether the specification complies with the written description requirement calls for the examiner to compare the scope of the claim with the scope of the description to determine whether applicant has demonstrated possession of the claimed invention. Such a review is conducted from the standpoint of one of skill in the art at the time the application was filed³⁴ and should include a determination of the field of the invention and the level of skill and knowledge in the art. Generally, there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. Information which is well known in the art need not be described in detail in the specification.³⁵

3. Determine Whether There is Sufficient Written Description to Inform a Skilled Artisan That Applicant was in Possession of the Claimed Invention as a Whole at the Time the Application Was Filed

a. Original claims. Possession may be shown in many ways. For example, possession may be shown, *inter alia*, by describing an actual reduction to practice of the claimed invention. Possession may also be shown by a clear depiction of the invention in detailed drawings or in structural chemical formulas which permit a person skilled in the art to clearly recognize that applicant had possession of the claimed invention. An adequate written description of the invention may be shown by any description of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention.³⁶

A specification may describe an actual reduction to practice by showing

that the inventor constructed an embodiment or performed a process that met all the limitations of the claim and determined that the invention would work for its intended purpose.³⁷ Description of an actual reduction to practice of a biological material may be shown by specifically describing a deposit made in accordance with the requirements of 37 CFR 1.801 *et seq.*³⁸

An applicant may show possession of an invention by disclosure of drawings³⁹ or structural chemical formulas⁴⁰ that are sufficiently detailed to show that applicant was in possession of the claimed invention as a whole. The description need only describe in detail that which is new or not conventional.⁴¹ This is equally true whether the claimed invention is directed to a product or a process.

An applicant may also show that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics⁴² which provide evidence that applicant was in possession of the claimed invention,⁴³ *i.e.*, complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics.⁴⁴ What is conventional or well known to one of ordinary skill in the art need not be disclosed in detail.⁴⁵ If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met.⁴⁶

(1) For each claim drawn to a single embodiment or species:⁴⁷

(a) Determine whether the application describes an actual reduction to practice of the claimed invention.

(b) If the application does not describe an actual reduction to practice, determine whether the invention is complete as evidenced by a reduction to drawings or structural chemical formulas that are sufficiently detailed to show that applicant was in possession of the claimed invention as a whole.

(c) If the application does not describe an actual reduction to practice or reduction to drawings or structural chemical formula as discussed above, determine whether the invention has been set forth in terms of distinguishing identifying characteristics as evidenced by other descriptions of the invention that are sufficiently detailed to show that applicant was in possession of the claimed invention.

(i) Determine whether the application as filed describes the complete structure

(or acts of a process) of the claimed invention as a whole. The complete structure of a species or embodiment typically satisfies the requirement that the description be set forth "in such full, clear, concise, and exact terms" to show possession of the claimed invention.⁴⁸ If a complete structure is disclosed, the written description requirement is satisfied for that species or embodiment, and a rejection under 35 U.S.C. 112, § 1, for lack of written description must not be made.

(ii) If the application as filed does not disclose the complete structure (or acts of a process) of the claimed invention as a whole, determine whether the specification discloses other relevant identifying characteristics sufficient to describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize applicant was in possession of the claimed invention.⁴⁹

Whether the specification shows that applicant was in possession of the claimed invention is not a single, simple determination, but rather is a factual determination reached by considering a number of factors. Factors to be considered in determining whether there is sufficient evidence of possession include the level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention. Disclosure of any combination of such identifying characteristics that distinguish the claimed invention from other materials and would lead one of skill in the art to the conclusion that the applicant was in possession of the claimed species is sufficient.⁵⁰ Patents and printed publications in the art should be relied upon to determine whether an art is mature and what the level of knowledge and skill is in the art. In most technologies which are mature, and wherein the knowledge and level of skill in the art is high, a written description question should not be raised for original claims even if the specification discloses only a method of making the invention and the function of the invention.⁵¹ In contrast, for inventions in emerging and unpredictable technologies, or for inventions characterized by factors not reasonably predictable which are known to one of ordinary skill in the art, more evidence is required to show possession. For example, disclosure of only a method of making the invention and the function may not be sufficient to support a product claim other than a

product-by-process claim.⁵²

Furthermore, disclosure of a partial structure without additional characterization of the product may not be sufficient to evidence possession of the claimed invention.⁵³

Any claim to a species that does not meet the test described under at least one of (a), (b), or (c) must be rejected as lacking adequate written description under 35 U.S.C. 112, § 1.

(2) For each claim drawn to a genus:

The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice (see (1)(a), above), reduction to drawings (see (1)(b), above), or by disclosure of relevant, identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus (see (1)(c), above).⁵⁴

A "representative number of species" means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. On the other hand, there may be situations where one species adequately supports a genus.⁵⁵ What constitutes a "representative number" is an inverse function of the skill and knowledge in the art. Satisfactory disclosure of a "representative number" depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. For inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species *cannot* be achieved by disclosing only one species within the genus.⁵⁶ Description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces.⁵⁷ If a representative number of adequately described species are not disclosed for a genus, the claim to that genus must be rejected as lacking adequate written description under 35 U.S.C. 112, § 1.

b. New claims, amended claims, or claims asserting entitlement to the benefit of an earlier priority date or filing date under 35 U.S.C. 119, 120, or

365(c). The examiner has the initial burden of presenting evidence or reasoning to explain why persons skilled in the art would not recognize in the original disclosure a description of the invention defined by the claims.⁵⁸ However, when filing an amendment an applicant should show support in the original disclosure for new or amended claims.⁵⁹ To comply with the written description requirement of 35 U.S.C. 112, ¶ 1, or to be entitled to an earlier priority date or filing date under 35 U.S.C. 119, 120, or 365(c), each claim limitation must be expressly,⁶⁰ implicitly,⁶¹ or inherently⁶² supported in the originally filed disclosure.⁶³ Furthermore, each claim must include all elements which applicant has described as essential.⁶⁴

If the originally filed disclosure does not provide support for each claim limitation, or if an element which applicant describes as essential or critical is not claimed, a new or amended claim must be rejected under 35 U.S.C. 112, ¶ 1, as lacking adequate written description, or in the case of a claim for priority under 35 U.S.C. 119, 120, or 365(c), the claim for priority must be denied.

III. Complete Patentability Determination Under All Statutory Requirements and Clearly Communicate Findings, Conclusions, and Their Bases

The above only describes how to determine whether the written description requirement of 35 U.S.C. 112, ¶ 1, is satisfied. Regardless of the outcome of that determination, Office personnel must complete the patentability determination under all the relevant statutory provisions of title 35 of the U.S. Code.

Once Office personnel have concluded analysis of the claimed invention under all the statutory provisions, including 35 U.S.C. 101, 112, 102, and 103, they should review all the proposed rejections and their bases to confirm their correctness. Only then should any rejection be imposed in an Office action. The Office action should clearly communicate the findings, conclusions, and reasons which support them. When possible, the Office action should offer helpful suggestions on how to overcome rejections.

A. For Each Claim Lacking Written Description Support, Reject the Claim Under Section 112, ¶ 1, for Lack of Adequate Written Description

A description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary

has been presented by the examiner to rebut the presumption.⁶⁵ The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. The examiner has the initial burden of presenting by a preponderance of evidence why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims.⁶⁶ In rejecting a claim, the examiner must set forth express findings of fact regarding the above analysis which support the lack of written description conclusion. These findings should:

- (1) Identify the claim limitation at issue; and
- (2) Establish a *prima facie* case by providing reasons why a person skilled in the art at the time the application was filed would not have recognized that the inventor was in possession of the invention as claimed in view of the disclosure of the application as filed. A general allegation of "unpredictability in the art" is not a sufficient reason to support a rejection for lack of adequate written description.

When appropriate, suggest amendments to the claims which can be supported by the application's written description, being mindful of the prohibition against the addition of new matter in the claims or description.⁶⁷

B. Upon Reply by Applicant, Again Determine the Patentability of the Claimed Invention, Including Whether the Written Description Requirement Is Satisfied by Reperforming the Analysis Described Above in View of the Whole Record

Upon reply by applicant, before repeating any rejection under 35 U.S.C. 112, ¶ 1, for lack of written description, review the basis for the rejection in view of the record as a whole, including amendments, arguments, and any evidence submitted by applicant. If the whole record now demonstrates that the written description requirement is satisfied, do not repeat the rejection in the next Office action. If the record still does not demonstrate that the written description is adequate to support the claim(s), repeat the rejection under 35 U.S.C. 112, ¶ 1, fully respond to applicant's rebuttal arguments, and properly treat any further showings submitted by applicant in the reply. When a rejection is maintained, any affidavits relevant to the 112, ¶ 1, written description requirement,⁶⁸ must be thoroughly analyzed and discussed in the next Office action.

Dated: December 29, 2000.

Q. Todd Dickinson,

Under Secretary of Commerce for Intellectual Property and Director of the United States Patent and Trademark Office.

Endnotes

¹ See, e.g., *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1560, 19 USPQ2d 1111, 1114 (Fed. Cir. 1991).

² *In re Barker*, 559 F.2d 588, 592 n.4, 194 USPQ 470, 473 n.4 (CCPA 1977).

³ See *Regents of the University of California v. Eli Lilly*, 119 F.3d 1559, 1566, 43 USPQ2d 1398, 1404 (Fed. Cir. 1997), cert. denied, 523 U.S. 1089 (1998).

⁴ See, e.g., *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116. Much of the written description case law addresses whether the specification as originally filed supports claims not originally in the application. The issue raised in the cases is most often phrased as whether the original application provides "adequate support" for the claims at issue or whether the material added to the specification incorporates "new matter" in violation of 35 U.S.C. 132. The "written description" question similarly arises in the interference context, where the issue is whether the specification of one party to the interference can support the newly added claims corresponding to the count at issue, i.e., whether that party can "make the claim" corresponding to the interference count. See, e.g., *Martin v. Mayer*, 823 F.2d 500, 503, 3 USPQ2d 1333, 1335 (Fed. Cir. 1987).

In addition, early opinions suggest the Patent and Trademark Office was unwilling to find written descriptive support when the only description was found in the claims; however, this viewpoint was rejected. See *In re Koller*, 613 F.2d 819, 204 USPQ 702 (CCPA 1980) (original claims constitute their own description); accord *In re Gardner*, 475 F.2d 1389, 177 USPQ 396 (CCPA 1973); accord *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976) (accord). It is now well accepted that a satisfactory description may be in the claims or any other portion of the originally filed specification. These early opinions did not address the quality or specificity of particularity that was required in the description, i.e., how much description is enough.

⁵ *Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997).

⁶ An application specification may show actual reduction to practice by describing testing of the claimed invention or, in the case of biological materials, by specifically describing a deposit made in accordance with 37 CFR 1.801 et seq. See also *Deposit of Biological Materials for Patent Purposes, Final Rule*, 54 FR 34,864 (August 22, 1989) ("The requirement for a specific identification is consistent with the description requirement of the first paragraph of 35 U.S.C. 112, and to provide an antecedent basis for the biological material which either has been or will be deposited before the patent is granted." *Id.* at 34,876. "The description must be sufficient to permit verification that the deposited biological material is in fact that disclosed. Once the

patent issues, the description must be sufficient to aid in the resolution of questions of infringement." *Id.* at 34,880.). Such a deposit is not a substitute for a written description of the claimed invention. The written description of the deposited material needs to be as complete as possible because the examination for patentability proceeds solely on the basis of the written description. *See, e.g., In re Lundak*, 773 F.2d 1216, 227 USPQ 90 (Fed. Cir. 1985). *See also* 54 FR at 34,880 ("As a general rule, the more information that is provided about a particular deposited biological material, the better the examiner will be able to compare the identity and characteristics of the deposited biological material with the prior art.").

⁷ *Pfaff v. Wells Electronics, Inc.*, 525 U.S. 55, 68, 119 S.Ct. 304, 312, 48 USPQ2d 1641, 1647 (1998); *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

⁸ *See Amgen, Inc. v. Chugai Pharmaceutical*, 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991) (one must define a compound by "whatever characteristics sufficiently distinguish it").

⁹ A description requirement issue can arise for original claims (*see, e.g., Eli Lilly*, 119 F.3d 1559, 43 USPQ2d 1398) as well as new or amended claims. Most typically, the issue will arise in the context of determining whether new or amended claims are supported by the description of the invention in the application as filed (*see, e.g., In re Wright*, 866 F.2d 422, 9 USPQ2d 1649 (Fed. Cir. 1989)), whether a claimed invention is entitled to the benefit of an earlier priority date or effective filing date under 35 U.S.C. 119, 120, or 365(c) (*see, e.g., Tronzo v. Biomet, Inc.*, 156 F.3d 1154, 47 USPQ2d 1829 (Fed. Cir. 1998); *Fiers v. Revel*, 984 F.2d 1164, 25 USPQ2d 1601 (Fed. Cir. 1993); *In re Ziegler*, 992 F.2d 1197, 1200, 26 USPQ2d 1600, 1603 (Fed. Cir. 1993)), or whether a specification provides support for a claim corresponding to a count in an interference (*see, e.g., Fields v. Conover*, 443 F.2d 1386, 170 USPQ 276 (CCPA 1971)).

¹⁰ *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116 (Fed. Cir. 1991).

¹¹ *In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976) ("we are of the opinion that the PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims").

¹² *See* endnote 4.

¹³ For example, consider the claim "A gene comprising SEQ ID NO:1." A determination of what the claim as a whole covers may result in a conclusion that specific structures such as a promoter, a coding region, or other elements are included. Although all genes encompassed by this claim share the characteristic of comprising SEQ ID NO:1, there may be insufficient description of those specific structures (*e.g.*, promoters, enhancers, coding regions, and other regulatory elements) which are also included.

¹⁴ A biomolecule sequence described only by a functional characteristic, without any known or disclosed correlation between that function and the structure of the sequence, normally is not a sufficient identifying

characteristic for written description purposes, even when accompanied by a method of obtaining the claimed sequence. For example, even though a genetic code table would correlate a known amino acid sequence with a genus of coding nucleic acids, the same table cannot predict the native, naturally occurring nucleic acid sequence of a naturally occurring mRNA or its corresponding cDNA. *Cf. In re Bell*, 991 F.2d 781, 26 USPQ2d 1529 (Fed. Cir. 1993), and *In re Deuel*, 51 F.3d 1552, 34 USPQ2d 1210 (Fed. Cir. 1995) (holding that a process could not render the product of that process obvious under 35 U.S.C. 103). The Federal Circuit has pointed out that under United States law, a description that does not render a claimed invention obvious cannot sufficiently describe the invention for the purposes of the written description requirement of 35 U.S.C. 112. *Eli Lilly*, 119 F.3d at 1567, 43 USPQ2d at 1405.

Compare Fonar Corp. v. General Electric Co., 107 F.3d 1543, 1549, 41 USPQ2d 1801, 1805 (Fed. Cir. 1997) ("As a general rule, where software constitutes part of a best mode of carrying out an invention, description of such a best mode is satisfied by a disclosure of the functions of the software. This is because, normally, writing code for such software is within the skill of the art, not requiring undue experimentation, once its functions have been disclosed. * * * Thus, flow charts or source code listings are not a requirement for adequately disclosing the functions of software.").

¹⁵ *See, e.g., Fujikawa v. Wattanasin*, 93 F.3d 1559, 1571, 39 USPQ2d 1895, 1905 (Fed. Cir. 1996) (a "laundry list" disclosure of every possible moiety does not constitute a written description of every species in a genus because it would not "reasonably lead" those skilled in the art to any particular species); *In re Ruschig*, 379 F.2d 990, 995, 154 USPQ 118, 123 (CCPA 1967) ("If n-propylamine had been used in making the compound instead of n-butylamine, the compound of claim 13 would have resulted. Appellants submit to us, as they did to the board, an imaginary specific example patterned on specific example 6 by which the above butyl compound is made so that we can see what a simple change would have resulted in a specific supporting disclosure being present in the present specification. The trouble is that there is no such disclosure, easy though it is to imagine it.") (emphasis in original); *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1328, 56 USPQ2d 1481, 1487 (Fed. Cir. 2000) ("the specification does not clearly disclose to the skilled artisan that the inventors * * * considered the [] ratio to be part of their invention * * *. There is therefore no force to Purdue's argument that the written description requirement was satisfied because the disclosure revealed a broad invention from which the [later-filed] claims carved out a patentable portion").

¹⁶ 35 U.S.C. §§ 132 and 251. *See also In re Rasmussen*, 650 F.2d 1212, 1214, 211 USPQ 323, 326 (CCPA 1981). *See Manual of Patent Examining Procedure (MPEP)* §§ 2163.06–2163.07 (7th Ed., Rev. 1, Feb. 2000) for a more detailed discussion of the written description requirement and its relationship to new matter.

¹⁷ The claims as filed in the original specification are part of the disclosure and, therefore, if an application as originally filed contains a claim disclosing material not found in the remainder of the specification, the applicant may amend the specification to include the claimed subject matter. *In re Benno*, 768 F.2d 1340, 226 USPQ 683 (Fed. Cir. 1985).

¹⁸ *See, e.g., In re Lukach*, 442 F.2d 967, 169 USPQ 795 (CCPA 1971) (subgenus range was not supported by generic disclosure and specific example within the subgenus range); *In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972) (a subgenus is not necessarily described by a genus encompassing it and a species upon which it reads).

¹⁹ *In re Oda*, 443 F.2d 1200, 170 USPQ 260 (CCPA 1971). With respect to the correction of sequencing errors in applications disclosing nucleic acid and/or amino acid sequences, it is well known that sequencing errors are a common problem in molecular biology. *See, e.g., Peter Richter, Estimation of Errors in 'Raw' DNA Sequences: A Validation Study*, 8 Genome Research 251–59 (1998). If an application as filed includes sequence information and references a deposit of the sequenced material made in accordance with the requirements of 37 CFR § 1.801 *et seq.*, amendment may be permissible.

²⁰ Corrections of minor errors in the sequence may be possible based on the argument that one of skill in the art would have resequenced the deposited material and would have immediately recognized the minor error. Deposits made after the filing date can only be relied upon to provide support for the correction of sequence information if applicant submits a statement in compliance with 37 CFR § 1.804 stating that the biological material which is deposited is a biological material specifically defined in the application as filed.

²¹ *See, e.g., Gentry Gallery, Inc. v. Berkline Corp.*, 134 F.3d 1473, 45 USPQ2d 1498 (Fed. Cir. 1998) (claims to a sectional sofa comprising, *inter alia*, a console and a control means were held invalid for failing to satisfy the written description requirement where the claims were broadened by removing the location of the control means.); *Johnson Worldwide Associates v. Zeeco Corp.*, 175 F.3d 985, 993, 50 USPQ2d 1607, 1613 (Fed. Cir. 1999) (In *Gentry Gallery*, the "court's determination that the patent disclosure did not support a broad meaning for the disputed claim terms was premised on clear statements in the written description that described the location of a claim element—the 'control means'—as 'the only possible location' and that variations were 'outside the stated purpose of the invention.' *Gentry Gallery*, 134 F.3d at 1479, 45 USPQ2d at 1503. *Gentry Gallery*, then, considers the situation where the patent's disclosure makes crystal clear that a particular (*i.e.*, narrow) understanding of a claim term is an 'essential element of [the inventor's] invention.'"); *Tronzo v. Biomet*, 156 F.3d at 1158–59, 47 USPQ2d at 1833 (Fed. Cir. 1998) (claims to generic cup shape were not entitled to filing date of parent application which disclosed "conical cup" in view of the disclosure of the

parent application stating the advantages and importance of the conical shape.)

²² See *Gentry Gallery*, 134 F.3d at 1480, 45 USPQ2d at 1503; *In re Sus*, 306 F.2d 494, 504, 134 USPQ 301, 309 (CCPA 1962) ("[O]ne skilled in this art would not be taught by the written description of the invention in the specification that any 'aryl or substituted aryl radical' would be suitable for the purposes of the invention but rather that only certain aryl radicals and certain specifically substituted aryl radicals [i.e., aryl azides] would be suitable for such purposes.") (emphasis in original). A claim which omits matter disclosed to be essential to the invention as described in the specification or in other statements of record may also be subject to rejection under 35 U.S.C. 112, ¶ 1, as not enabling, or under 35 U.S.C. 112, ¶ 2. See *In re Mayhew*, 527 F.2d 1229, 188 USPQ 356 (CCPA 1976); *In re Venezia*, 530 F.2d 956, 189 USPQ 149 (CCPA 1976); and *In re Collier*, 397 F.2d 1003, 158 USPQ 266 (CCPA 1968). See also MPEP § 2172.01.

²³ See, e.g., *Vas-Cath, Inc.*, 935 F.2d at 1563-64, 19 USPQ2d at 1117.

²⁴ *Wertheim*, 541 F.2d at 262, 191 USPQ at 96.

²⁵ See MPEP §§ 714.02 and 2163.06 ("Applicant should * * * specifically point out the support for any amendments made to the disclosure."); and MPEP § 2163.04 ("If applicant amends the claims and points out where and/or how the originally filed disclosure supports the amendment(s), and the examiner finds that the disclosure does not reasonably convey that the inventor had possession of the subject matter of the amendment at the time of the filing of the application, the examiner has the initial burden of presenting evidence or reasoning to explain why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims.").

²⁶ See *In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972) ("Precisely how close [to the claimed invention] the description must come to comply with § 112 must be left to case-by-case development."); *In re Wertheim*, 541 F.2d at 262, 191 USPQ at 96 (inquiry is primarily factual and depends on the nature of the invention and the amount of knowledge imparted to those skilled in the art by the disclosure).

²⁷ See, e.g., *In re Morris*, 127 F.3d 1048, 1053-54, 44 USPQ2d 1023, 1027 (Fed. Cir. 1997).

²⁸ "Preamble language" is that language in a claim appearing before the transitional phrase, e.g., before "comprising," "consisting essentially of," or "consisting of."

²⁹ The transitional term "comprising" (and other comparable terms, e.g., "containing," "including," and "having") is "open-ended—it covers the expressly recited subject matter, alone or in combination with unrecited subject matter. See, e.g., *Genentech, Inc. v. Chiron Corp.*, 112 F.3d 495, 501, 42 USPQ2d 1608, 1613 (Fed. Cir. 1997) ("'Comprising' is a term of art used in claim language which means that the named elements are essential, but other elements may be added and still form a construct within the scope of the claim."); *Ex parte Davis*, 80 USPQ 448, 450 (Bd. App. 1948) ("comprising" leaves the

"claim open for the inclusion of unspecified ingredients even in major amounts"). "By using the term 'consisting essentially of,' the drafter signals that the invention necessarily includes the listed ingredients and is open to unlisted ingredients that do not materially affect the basic and novel properties of the invention. A 'consisting essentially of' claim occupies a middle ground between closed claims that are written in a 'consisting of' format and fully open claims that are drafted in a 'comprising' format." *PPG Industries v. Guardian Industries*, 156 F.3d 1351, 1354, 48 USPQ2d 1351, 1353-54 (Fed. Cir. 1998). For the purposes of searching for and applying prior art under 35 U.S.C. 102 and 103, absent a clear indication in the specification or claims of what the basic and novel characteristics actually are, "consisting essentially of" will be construed as equivalent to "comprising." See, e.g., *PPG*, 156 F.3d at 1355, 48 USPQ2d at 1355 ("PPG could have defined the scope of the phrase 'consisting essentially of' for purposes of its patent by making clear in its specification what it regarded as constituting a material change in the basic and novel characteristics of the invention."). See also *In re Janakirama-Rao*, 317 F.2d 951, 954, 137 USPQ 893, 895-96 (CCPA 1963). If an applicant contends that additional steps or materials in the prior art are excluded by the recitation of "consisting essentially of," applicant has the burden of showing that the introduction of additional steps or components would materially change the characteristics of applicant's invention. *In re De Lajarte*, 337 F.2d 870, 143 USPQ 256 (CCPA 1964).

³⁰ See *Pac-Tec Inc. v. Amerace Corp.*, 903 F.2d 796, 801, 14 USPQ2d 1871, 1876 (Fed. Cir. 1990) (determining that preamble language that constitutes a structural limitation is actually part of the claimed invention).

³¹ An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations. *Lockwood*, 107 F.3d at 1572, 41 USPQ2d at 1966.

³² See, e.g., *Bell Communications Research, Inc. v. Vitalink Communications Corp.*, 55 F.3d 615, 620, 34 USPQ2d 1816, 1820 (Fed. Cir. 1995) ("[A] claim preamble has the import that the claim as a whole suggests for it."); *Corning Glass Works v. Sumitomo Elec. U.S.A., Inc.*, 868 F.2d 1251, 1257, 9 USPQ2d 1962, 1966 (Fed. Cir. 1989) (The determination of whether preamble recitations are structural limitations can be resolved only on review of the entirety of the application "to gain an understanding of what the inventors actually invented and intended to encompass by the claim.").

³³ An element may be critical where those of skill in the art would require it to determine that applicant was in possession of the invention. *Compare Rasmussen*, 650 F.2d at 1215, 211 USPQ at 327 ("one skilled in the art who read Rasmussen's specification would understand that it is unimportant how the layers are adhered, so long as they are adhered") (emphasis in original), with *Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd.*, 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991) ("it is well established in our law that conception of a chemical

compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it").

³⁴ See, e.g., *Wang Labs. v. Toshiba Corp.*, 993 F.2d 858, 865, 26 USPQ2d 1767, 1774 (Fed. Cir. 1993).

³⁵ See, e.g., *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986).

³⁶ See, e.g., *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, ___, 56 USPQ2d 1481, 1483 (Fed. Cir. 2000) (the written description "inquiry is a factual one and must be assessed on a case-by-case basis"); see also *Pfaff v. Wells Electronics, Inc.*, 55 U.S. at 66, 119 S.Ct. at 311, 48 USPQ2d at 1646 ("The word 'invention' must refer to a concept that is complete, rather than merely one that is 'substantially complete.' It is true that reduction to practice ordinarily provides the best evidence that an invention is complete. But just because reduction to practice is sufficient evidence of completion, it does not follow that proof of reduction to practice is necessary in every case. Indeed, both the facts of the *Telephone Cases* and the facts of this case demonstrate that one can prove that an invention is complete and ready for patenting before it has actually been reduced to practice.").

³⁷ *Cooper v. Goldfarb*, 154 F.3d 1321, 1327, 47 USPQ2d 1896, 1901 (Fed. Cir. 1998). See also *UMC Elecs. Co. v. United States*, 816 F.2d 647, 652, 2 USPQ2d 1465, 1468 (Fed. Cir. 1987) ("[T]here cannot be a reduction to practice of the invention * * * without a physical embodiment which includes all limitations of the claim."); *Estee Lauder Inc. v. L'Oreal, S.A.*, 129 F.3d 588, 593, 44 USPQ2d 1610, 1614 (Fed. Cir. 1997) ("[A] reduction to practice does not occur until the inventor has determined that the invention will work for its intended purpose."); *Mahurkar v. C.R. Bard, Inc.*, 79 F.3d 1572, 1578, 38 USPQ2d 1288, 1291 (Fed. Cir. 1996) (determining that the invention will work for its intended purpose may require testing depending on the character of the invention and the problem it solves).

³⁸ 37 CFR 1.804, 1.809. See also endnote 6.

³⁹ See, e.g., *Vas-Cath*, 935 F.2d at 1565, 19 USPQ2d at 1118 ("drawings alone may provide a 'written description' of an invention as required by § 112"); *In re Wolfensperger*, 302 F.2d 950, 133 USPQ 537 (CCPA 1962) (the drawings of applicant's specification provided sufficient written descriptive support for the claim limitation at issue); *Autogiro Co. of America v. United States*, 384 F.2d 391, 398, 155 USPQ 697, 703 (Ct. Cl. 1967) ("In those instances where a visual representation can flesh out words, drawings may be used in the same manner and with the same limitations as the specification.").

⁴⁰ See, e.g., *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406 ("In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus.").

⁴¹ See *Hybritech v. Monoclonal Antibodies*, 802 F.2d at 1384, 231 USPQ at 94; *Fonar Corp. v. General Electric Co.*, 107 F.3d at 1549, 41 USPQ2d at 1805 (source code description not required).

⁴² For example, the presence of a restriction enzyme map of a gene may be relevant to a statement that the gene has been isolated. One skilled in the art may be able to determine when the gene disclosed is the same as or different from a gene isolated by another by comparing the restriction enzyme map. In contrast, evidence that the gene could be digested with a nuclease would not normally represent a relevant characteristic since any gene would be digested with a nuclease. Similarly, isolation of an mRNA and its expression to produce the protein of interest is strong evidence of possession of an mRNA for the protein.

For some biomolecules, examples of identifying characteristics include a sequence, structure, binding affinity, binding specificity, molecular weight, and length. Although structural formulas provide a convenient method of demonstrating possession of specific molecules, other identifying characteristics or combinations of characteristics may demonstrate the requisite possession. For example, unique cleavage by particular enzymes, isoelectric points of fragments, detailed restriction enzyme maps, a comparison of enzymatic activities, or antibody cross-reactivity may be sufficient to show possession of the claimed invention to one of skill in the art. See *Lockwood*, 107 F.3d at 1572, 41 USPQ2d at 1666 ("written description" requirement may be satisfied by using "such descriptive means as words, structures, figures, diagrams, formulas, etc., that fully set forth the claimed invention").

⁴³ A definition by function alone "does not suffice" to sufficiently describe a coding sequence "because it is only an indication of what the gene does, rather than what it is." *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406. See also *Fiers*, 984 F.2d at 1169-71, 25 USPQ2d at 1605-06 (discussing *Amgen Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 18 USPQ2d 1016 (Fed. Cir. 1991)).

⁴⁴ If a claim limitation invokes 35 U.S.C. 112, ¶ 6, it must be interpreted to cover the corresponding structure, materials, or acts in the specification and "equivalents thereof." See 35 U.S.C. 112, ¶ 6. See also *B. Braun Medical, Inc. v. Abbott Lab.*, 124 F.3d 1419, 1424, 43 USPQ2d 1896, 1899 (Fed. Cir. 1997). In considering whether there is 35 U.S.C. 112, ¶ 1, support for a means- (or step-) plus-function claim limitation, the examiner must consider not only the original disclosure contained in the summary and detailed description of the invention portions of the specification, but also the original claims, abstract, and drawings. A means- (or step-) plus-function claim limitation is adequately described under 35 U.S.C. 112, ¶ 1, if: (1) The written description adequately links or associates adequately described particular structure, material, or acts to the function recited in a means- (or step-) plus-function claim limitation; or (2) it is clear based on the facts of the application that one skilled in the art would have known what structure, material, or acts perform the function recited in a means- (or step-) plus-

function limitation. Note also: A rejection under 35 U.S.C. 112, ¶ 2, "cannot stand where there is adequate description in the specification to satisfy 35 U.S.C. 112, first paragraph, regarding means-plus-function recitations that are not, per se, challenged for being unclear." *In re Noll*, 545 F.2d 141, 149, 191 USPQ 721, 727 (CCPA 1976). See *Supplemental Examination Guidelines for Determining the Applicability of 35 U.S.C. 112*, ¶ 6, 65 FR 38510, June 21, 2000.

⁴⁵ See *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d at 1384, 231 USPQ at 94.

⁴⁶ See, e.g., *Vas-Cath*, 935 F.2d at 1563, 19 USPQ2d at 1116; *Martin v. Johnson*, 454 F.2d 746, 751, 172 USPQ 391, 395 (CCPA 1972) (stating "the description need not be in *ipsis verbis* [i.e., "in the same words"] to be sufficient").

⁴⁷ A claim which is limited to a single disclosed embodiment or species is analyzed as a claim drawn to a single embodiment or species, whereas a claim which encompasses two or more embodiments or species within the scope of the claim is analyzed as a claim drawn to a genus. See also MPEP § 806.04(e).

⁴⁸ 35 U.S.C. 112, ¶ 1. *Cf. Fields v. Conover*, 443 F.2d 1386, 1392, 170 USPQ 276, 280 (CCPA 1971) (finding a lack of written description because the specification lacked the "full, clear, concise, and exact written description" which is necessary to support the claimed invention).

⁴⁹ For example, if the art has established a strong correlation between structure and function, one skilled in the art would be able to predict with a reasonable degree of confidence the structure of the claimed invention from a recitation of its function. Thus, the written description requirement may be satisfied through disclosure of function and minimal structure when there is a well-established correlation between structure and function. In contrast, without such a correlation, the capability to recognize or understand the structure from the mere recitation of function and minimal structure is highly unlikely. In this latter case, disclosure of function alone is little more than a wish for possession; it does not satisfy the written description requirement. See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406 (written description requirement not satisfied by merely providing "a result that one might achieve if one made that invention"); *In re Wilder*, 736 F.2d 1516, 1521, 222 USPQ 369, 372-73 (Fed. Cir. 1984) (affirming a rejection for lack of written description because the specification does "little more than outline goals appellants hope the claimed invention achieves and the problems the invention will hopefully ameliorate"). Compare *Fonar*, 107 F.3d at 1549, 41 USPQ2d at 1805 (disclosure of software function adequate in that art).

⁵⁰ See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

⁵¹ See, e.g., *In re Hayes Microcomputer Products, Inc. Patent Litigation*, 982 F.2d 1527, 1534-35, 25 USPQ2d 1241, 1246 (Fed. Cir. 1992) ("One skilled in the art would know how to program a microprocessor to perform the necessary steps described in the specification. Thus, an inventor is not required to describe every detail of his invention. An applicant's disclosure

obligation varies according to the art to which the invention pertains. Disclosing a microprocessor capable of performing certain functions is sufficient to satisfy the requirement of section 112, first paragraph, when one skilled in the relevant art would understand what is intended and know how to carry it out.")

⁵² See, e.g., *Fiers v. Revel*, 984 F.2d at 1169, 25 USPQ2d at 1605; *Amgen*, 927 F.2d at 1206, 18 USPQ2d at 1021. Where the process has actually been used to produce the product, the written description requirement for a product-by-process claim is clearly satisfied; however, the requirement may not be satisfied where it is not clear that the acts set forth in the specification can be performed, or that the product is produced by that process.

⁵³ See, e.g., *Amgen*, 927 F.2d at 1206, 18 USPQ2d at 1021 ("A gene is a chemical compound, albeit a complex one, and it is well established in our law that conception of a chemical compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it. Conception does not occur unless one has a mental picture of the structure of the chemical, or is able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics sufficiently distinguish it. It is not sufficient to define it solely by its principal biological property, e.g., encoding human erythropoietin, because an alleged conception having no more specificity than that is simply a wish to know the identity of any material with that biological property. We hold that when an inventor is unable to envision the detailed constitution of a gene so as to distinguish it from other materials, as well as a method for obtaining it, conception has not been achieved until reduction to practice has occurred, i.e., until after the gene has been isolated.") (citations omitted). In such instances the alleged conception fails not merely because the field is unpredictable or because of the general uncertainty surrounding experimental sciences, but because the conception is incomplete due to factual uncertainty that undermines the specificity of the inventor's idea of the invention. *Burroughs Wellcome Co. v. Barr Laboratories Inc.*, 40 F.3d 1223, 1229, 32 USPQ2d 1915, 1920 (Fed. Cir. 1994). Reduction to practice in effect provides the only evidence to corroborate conception (and therefore possession) of the invention. *Id.*

⁵⁴ See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

⁵⁵ See, e.g., *Rasmussen*, 650 F.2d at 1214, 211 USPQ at 326-27 (disclosure of a single method of adheringly applying one layer to another was sufficient to support a generic claim to "adheringly applying" because one skilled in the art reading the specification would understand that it is unimportant how the layers are adhered, so long as they are adhered). *In re Herschler*, 591 F.2d 693, 697, 200 USPQ 711, 714 (CCPA 1979) (disclosure of corticosteroid in DMSO sufficient to support claims drawn to a method of using a mixture of a "physiologically active steroid" and DMSO because "use of known chemical compounds in a manner auxiliary

to the invention must have a corresponding written description only so specific as to lead one having ordinary skill in the art to that class of compounds. Occasionally, a functional recitation of those known compounds in the specification may be sufficient as that description."'); *In re Smythe*, 480 F.2d 1376, 1383, 178 USPQ 279, 285 (CCPA 1973) (the phrase "air or other gas which is inert to the liquid" was sufficient to support a claim to "inert fluid media" because the description of the properties and functions of the air or other gas segmentizing medium would suggest to a person skilled in the art that appellant's invention includes the use of "inert fluid" broadly.). However, in *Tronzo v. Biomet*, 156 F.3d at 1159, 47 USPQ2d at 1833 (Fed. Cir. 1998), the disclosure of a species in the parent application did not suffice to provide written description support for the genus in the child application.

⁵⁶ See, e.g., *Eli Lilly*.

⁵⁷ For example, in the molecular biology arts, if an applicant disclosed an amino acid sequence, it would be unnecessary to provide an explicit disclosure of nucleic acid sequences that encoded the amino acid sequence. Since the genetic code is widely known, a disclosure of an amino acid sequence would provide sufficient information such that one would accept that an applicant was in possession of the full genus of nucleic acids encoding a given amino acid sequence, but not necessarily any particular species. Cf. *In re Bell*, 991 F.2d 781, 785, 26 USPQ2d 1529, 1532 (Fed. Cir. 1993) and *In re Baird*, 16 F.3d 380, 382, 29 USPQ2d 1550, 1552 (Fed. Cir. 1994).

⁵⁸ See *Wertheim*, 541 F.2d at 263, 191 USPQ at 97 ("[T]he PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims.").

⁵⁹ See MPEP §§ 714.02 and 2163.06 ("Applicant should * * * specifically point out the support for any amendments made to the disclosure.").

⁶⁰ See, e.g., *In re Wright*, 866 F.2d 422, 425, 9 USPQ2d 1649, 1651 (Fed. Cir. 1989) (Original specification for method of forming images using photosensitive microcapsules which describes removal of microcapsules from surface and warns that capsules not be disturbed prior to formation of image, unequivocally teaches absence of permanently fixed microcapsules and supports amended language of claims requiring that microcapsules be "not permanently fixed" to underlying surface, and therefore meets description requirement of 35 U.S.C. 112.).

⁶¹ See, e.g., *In re Robins*, 429 F.2d 452, 456-57, 166 USPQ 552, 555 (CCPA 1970) ("[W]here no explicit description of a generic invention is to be found in the specification * * * mention of representative compounds may provide an implicit description upon which to base generic claim language."); *In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972) (a subgenus is not necessarily implicitly described by a genus encompassing it and a species upon which it reads).

⁶² See, e.g., *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir.

1999) ("To establish inherency, the extrinsic evidence "must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient."") (citations omitted).

⁶³ When an explicit limitation in a claim "is not present in the written description whose benefit is sought it must be shown that a person of ordinary skill would have understood, at the time the patent application was filed, that the description requires that limitation." *Hyatt v. Boone*, 146 F.3d 1348, 1353, 47 USPQ2d 1128, 1131 (Fed. Cir. 1998).

⁶⁴ See, e.g., *Johnson Worldwide Associates Inc. v. Zebco Corp.*, 175 F.3d at 993, 50 USPQ2d at 1613; *Gentry Gallery, Inc. v. Berkline Corp.*, 134 F.3d at 1479, 45 USPQ2d at 1503; *Tronzo v. Biomet*, 156 F.3d at 1159, 47 USPQ2d at 1833.

⁶⁵ See, e.g., *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971).

⁶⁶ *Wertheim*, 541 F.2d at 263, 191 USPQ at 97.

⁶⁷ See *Rasmussen*, 650 F.2d at 1214, 211 USPQ at 326.

⁶⁸ See *In re Alton*, 76 F.3d 1168, 1176, 37 USPQ2d 1578, 1584 (Fed. Cir. 1996).

[FR Doc. 01-323 Filed 1-4-01; 8:45 am]

BILLING CODE 3510-16-U

CORPORATION FOR NATIONAL AND COMMUNITY SERVICE

Revision of Currently Approved Information Collection; Comment Request

AGENCY: Corporation for National and Community Service

ACTION: Notice.

SUMMARY: The Corporation for National and Community Service (hereinafter "Corporation"), as part of its continuing effort to reduce paperwork and respondent burden, conducts a preclearance consultation program to provide the general public and Federal agencies with an opportunity to comment on proposed and/or continuing collections of information in accordance with the Paperwork Reduction Act of 1995 (PRA95) (44 U.S.C. 3506(c)(2)(A)). This program helps to ensure that requested data can be provided in the desired format, reporting burden (time and financial resources) is minimized, collection instruments are clearly understood, and the impact of collection requirement on respondents can be properly assessed.

Currently, the Corporation is soliciting comments concerning the proposed revision of its Voucher and

Payment Request Form (OMB #3045-0014).

Copies of the forms can be obtained by contacting the office listed below in the address section of this notice.

DATES: Written comments must be submitted to the office listed in the ADDRESSES section by March 6, 2001.

ADDRESSES: Send comments to Levon Buller, National Service Trust, Corporation for National and Community Service, 1201 New York Ave., NW., Washington, DC 20525.

FOR FURTHER INFORMATION CONTACT: Levon Buller, (202) 606-5000, ext. 383.

SUPPLEMENTARY INFORMATION: The Corporation is particularly interested in comments which:

- Evaluate whether the proposed collection of information is necessary for the proper performance of the functions of the Corporation, including whether the information will have practical utility;
- Evaluate the accuracy of the agency's estimate of the burden of the proposed collection of information, including the validity of the methodology and assumptions used;
- Enhance the quality, utility and clarity of the information to be collected; and
- Minimize the burden of the collection of information on those who are to respond, including through the use of appropriate automated, electronic, mechanical, or other technological collection techniques or other forms of information technology, e.g., permitting electronic submissions of responses.

Background

The Corporation supports programs that provide opportunities for individuals who want to become involved in national service. The service opportunities cover a wide range of activities over varying periods of time. Upon successfully completing an agreed-upon term of service in an approved AmeriCorps program, a national service participant—an AmeriCorps member—receives an "education award". This award is an amount of money set aside in the member's name in the National Service Trust Fund. This education award can be used to make payments towards qualified student loan or pay for educational expenses at qualified post-secondary institutions and approved school-to-work opportunities programs. Members have seven years in which to draw against any unused balance.

The National Service Trust is the office within the Corporation that administers the education award

PEPTIDE MOTIF REGISTER

Kirsten Falk · Olaf Röttschke · Masafumi Takiguchi
Blazenka Grahovac · Volker Gnau · Stefan Stevanović
Günther Jung · Hans-Georg Rammensee

Peptide motifs of HLA-A1, -A11, -A31, and -A33 molecules

Received: 7 March 1994 / Revised: 11 April 1994

As a source of HLA-A molecules, the human B-cell line C1R (Alexander et al. 1990; Zemmour et al. 1992) transfected with the respective genes was used. C1R cells do not express HLA-A or -B molecules on their own, with the exception of small amounts of HLA-B35 (Zemmour et al. 1992). In addition, C1R cells express HLA-Cw4. C1R-A1 cells were a gift of P. Romero, Lausanne; C1R-A11 (A*1101), C1R-A31 (A*3101), and C1R-A33 (A*3302; Kato et al. 1993), each highly expressing the respective molecules, were produced at the University of Tokyo. HLA-A*1101 and A*3101 genes were previously cloned from Japanese individuals and sequenced (data not shown). Transfectants were grown to about 10^{10} to 10^{11} cells in roller bottle tissue culture. HLA molecules were precipitated from detergent lysates of the cells, using solid-phase bound antibodies specific for HLA-A, -B, -C, PA2.6 (Parham and Bodmer 1978), or W6/32 (Barnstable et al. 1978), as described (Falk et al. 1991). Precipitates were treated with trifluoroacetic acid to dissociate peptides, and the peptides were separated from the remaining material by reversed phase high-performance liquid chromatography, using the SMART system (Pharmacia, Freiburg, Germany),

equipped with a 2.1 mm × 100 mm C2/C18 column. In some experiments, peptides were separated on a 4.0 mm × 250 mm C2/C18 column. The material eluting in larger peaks was collected and sequenced separately, whereas the remaining peptide fractions were pooled and sequenced as a mixture. Since the antibodies used for precipitation recognize HLA-B35 and HLA-Cw4, both expressed by C1R cells in small amounts, in addition to the transfected HLA-A, some contribution of B35 and Cw4 ligands to the peptide pools is to be expected. Previous results have shown, however, that this contribution is small, at best (Röttschke et al. 1994), and since both the B35 and the Cw4 peptide motifs are known (Falk et al. 1993a, b), this contribution can be judged to be negligible for the HLA-A motifs shown below.

Pool sequencing of A1 ligands indicated a decent peptide motif, calling for mainly nonameric peptide ligands (Fig. 1). The only residue found at position 9 is a Tyr, indicating this aromatic residue to be an anchor at the C-terminus of ligands. Tailing of the Tyr signal in subsequent cycles suggested the presence of peptides longer than 9 amino acids and terminated by Tyr as well. Another anchor is at position 3, occupied exclusively by the negatively charged residues Asp and Glu. Position 2 shows a strong but not exclusive preference for Thr and Ser, both having small side chains with OH-groups. Position 7 is preferentially occupied by Leu. Thus, position 3 and 9 are anchors, and 2 and 7 are auxiliary anchors. Very similar conclusions were drawn recently by Di Brino and co-workers (1993b, 1994).

Eight individual ligands eluting in major peaks were able to be sequenced (Fig. 1). Five ligands consist of 9 amino acids, two of 12, and one of 13 residues, and all have Tyr at the C-terminal position. Seven of these ligands use Asp or Glu at P3, thus confirming the pool sequencing motif. Arg 156, located at the bottom of the D-pocket in A1-molecules, is likely to provide the positive counter charge for the carboxyl groups of the P3 anchor side chain (Di Brino et al. 1994). The reverse effect has been observed in the case of HLA-B8, where a negatively charged Asp 156 residue is correlated with a preference for positively

K. Falk · O. Röttschke
Harvard University, Dept. of Biochemistry and Molecular Biology,
7 Divinity Avenue, Cambridge, MA 02138, USA

M. Takiguchi
The University of Tokyo, The Institute of Medical Science,
Dept. of Tumor Biology, 4-6-1 Shirokanedai Minato-ku, Tokyo, Japan

B. Grahovac
Institute of Clinical Laboratory Diagnostics, Clinical Center,
Zagreb University School of Medicine, Kispaticeva 12, 41000 Zagreb,
Croatia

V. Gnau · G. Jung
Universität Tübingen, Institut für Organische Chemie,
Auf der Morgenstelle 18, 72076 Tübingen, Germany

S. Stevanović · H.-G. Rammensee (✉)
Deutsches Krebsforschungszentrum,
Abteilung Tumovirus-Immunologie 0620, Im Neuenheimer Feld 242,
69120 Heidelberg, Germany

Fig. 1 HLA-A1 motif. **Bold** symbols indicate anchor position and residues. **Underlined** numbers indicate auxiliary anchor positions. Judgement of pool sequencing data and classification of anchor and other residues was done as described in Falk and co-workers (1991)

	Position											HLA-A1
	1	2	3	4	5	6	7	8	9			
Anchor or auxiliary anchor residues		T	D					L	Y			
		S	E									
Other preferred residues		L		P	G	G						Source
				G	N	V						
				I	Y	I						
Examples for ligands	A	I	D	F	K	F	A	M	Y			Cyclin-like protein
	I	A	D	M	G	H	L	K	Y			Proliferation cell nuclear antigen
	M	I	E	P	R	T	L	Q	Y			Ribosomal protein S16
	Y	I	S	D	Y	F	I	S	Y			Ets-1
	L	T	D	P	G	V	L	D	Y			Unknown
	V	S	D	I	V	G	P	D	G	L	V	Fibrillarin
	Y	T	D	Y	G	G	L	I	F	N	S	Cytochrome C oxidase II
	Q	S	E	D	G	S	H	T	I	Q	I	HLA class I α chain
T-cell epitopes	E	A	D	P	T	G	H	S	Y			MAGE1 (Van der Bruggen et al. 1991)
	V	S	D	G	G	P	N	L	Y			Influenza basic polymerase 1 (Di Brino et al. 1993 b)

Fig. 2 HLA-A11 motif

	Position											HLA-A11
	1	2	3	4	5	6	7	8	9	10	11	
Anchor or auxiliary anchor residues		V	M					L	K	K	K	
		I	L					I				
		F	F					Y				
		Y	Y					V				
			I					F				
			A									
Other preferred residues	A	T	N	P	P	I		R	R	R	R	
			D	G	I	V		K	D			
			E	D	F	M		N				
			Q		E	V		E				
				K	M			Q				Source
Examples for ligands	A	V	M	K	P	E	A	E	K	R	K	Unknown
	A	V	I	L	P	P	L	S	P	Y	F	HSB 66 EST
	A	S	E	D	K	A	K	L	K	K		Thymosin β -10
	G	Q	Y	G	N	P	L	N	K			Bovine metalloproteinase
	G	V	M	P	S	H	E	S	R			Ribosomal protein S19
	Y	E	D	P	A	N	G	K	F	S	K	Elongation factor 2
	A	T	A	G	D	G	L	I	E	L	R	Prohibitin (rat)
	S	I	Y	Y	G	S	E	V	T	R		Unknown
T-cell epitope	I	V	T	D	F	S	V	I	K			EBNA 4 (Zhang et al. 1993; Gavioli et al. 1993)

charged P3 side chains (Sutton et al. 1993; Malcherek et al. 1993; Di Brino et al. 1994). Six of these eight peptides use the auxiliary anchor at P2 (Thr or Ser), five use either Leu or the closely related Ile at P7. One ligand having neither Asp nor Glu at P3 has Asp at P4, suggesting that in this peptide the anchor site might be shifted by one position, especially since both auxiliary anchors (P2 with Thr and P7 with Ile instead of Leu) are used. Seven of these peptides were identified as being derived from normal cellular proteins. One ligand is derived from a mitochondrial protein, cytochrome C oxidase II, and thus represents another example of a mitochondrial protein being a peptide donor for a class I molecule, in addition to NADH-dehydrogenase (Loveland et al. 1990) and 2-oxoglutarate dehydrogenase (Udaka et al. 1993). An optimal T-cell epitope (not shown so far to be a natural A1 ligand) representing a melanoma-associated antigen, MAGE1 (Van der Bruggen et al. 1991), and another one from influenza (Di Brino et al. 1993 b) also fit well to the motif.

Most significant for the pool sequencing data of A11 ligands (Fig. 2) were strong signals for positively charged residues around P9, mainly Lys and to a lesser extent Arg. A strong preference for hydrophobic residues was evident at P2 or P3, as well as at P7. The eight individual ligands that could be sequenced are between 9 and 12 residues long, indicating that A11 molecules can accommodate

peptides consisting of 9 to 12 amino acids, whereby the C-terminus is either Arg or Lys. This notion was also confirmed by the sequencing results of several HPLC peaks containing multiple sequence signals while showing strong Lys or, less frequently, Arg signals at P9 to P12. P2 and P3 as well as P7 are auxiliary anchors for hydrophobic residues. The various length of peptides among A11 ligands is reminiscent of the Aw68 specificity; the latter molecule can accommodate peptides of different numbers of residues by bulging out the central residues of the longer peptides (Guo et al. 1992). An A11-restricted T-cell epitope of Epstein-Barr virus (EBNA4; Zhang et al. 1993; Gavioli et al. 1993) fits well to the motif. A previously reported A11-specific peptide binding motif (Zhang et al. 1993) is well compatible with our natural ligand motif.

Similar to A11, A31 molecules carry peptides with a basic C-terminus; in contrast to A11, however, nonamers with a C-terminal Arg are preferred (Fig. 3). Other conserved positions are P2, P3, and P6, preferring hydrophobic residues. Thus, A31 molecules require ligands with a positively charged C-terminus and no additional clearcut anchor but with P2, P3, and P6 as auxiliary anchors. Six individual ligands could be identified that confirm the motif length (4 nonamers, one decamer, one undecamer), as well as the C-terminal anchor and the hydrophobic auxiliary anchor in P3, whereas the other auxiliary anchors are not

Fig. 3 HLA-A31 motif

	1	2	3	4	5	6	7	8	9	HLA-A31
Anchor or auxiliary anchor residues		L V Y F	F L Y W			L F V I			R	
Other preferred residues	K	T Q	K N	P D E G S V T	P I V F L Y W	T N D E R T H L	N V R N Q	L		
Examples for ligands	L Q R K K R	Q Q G V I Y	E L Y E M D	P Y R G K D	V W P P I W	G S H H I A	R H P R F H E N T	V P R R E Y S	H R R R R R	R
T-cell epitope	S	T	L	P	E	T	T	V	V	R R
Source										Histon H2 a 5 Ribosomal protein S29 (rat) CCAAT-binding transcription factor [GlcNac]-P-transferase Unknown Lamin B2 Hepatitis BcAg (Missale et al. 1993)

Fig. 4 HLA-A33 motif

	1	2	3	4	5	6	7	8	9	HLA-A33
Anchor or auxiliary anchor residues		A I L F Y Y V							R	
Preferred residues		T	L K	P	P	I L F				
Other possible residues	E M		Q W E N	R D E G H P	R I F P L W	R D H Y T Q	H Y V S	Q N E M		
Examples for ligands	D E T D E T	M S Y Y I I	A G Y H M M	A P G I K P	Q S S W K D	I I F N R D	T V V R E I	Q H T Q R L	R R R R R	R
Source										HLA class I α -chain Actin Unknown Human cDNA HSB15F102 Unknown Histon 3.1/3.3

frequently used. A T-cell epitope of Hepatitis B virus (Missale et al. 1993) fits well to the motif.

Pool sequencing of A33 ligands again indicates a high frequency of nonamers with a positively charged C-terminal anchor dominated by Arg (Fig. 4). P2 is preferentially occupied by a variety of hydrophobic or aromatic residues. These aspects of the A33 motif are well reflected in the six individual ligands. Four of them are nonamers; all have Arg at the C-terminus, and five of these peptides have one of the hydrophobic or aromatic residues found at P2 of the pool sequence, confirming P2 as an auxiliary anchor.

The side chains of C-terminal residues of class I ligands are accommodated in the F-pocket of the groove (Saper et al. 1991; Madden et al. 1991; Matsamura et al. 1992). X-ray crystallography of B27 and Aw68 crystals indicate bonds between Asp 116 and Arg or Lys side chains of P9 residues (Madden et al. 1991; Silver et al. 1992). A comparison of the amino acids contributing to the F-pocket in

various class I sequences indicated that position 116 is always an Asp in those class I molecules accommodating peptides with positively charged C-terminal residues (Falk and Rötzschke 1993). The 4 class I molecules investigated in the present paper all possess Asp at 116 (Bjorkman and Parham 1990). Thus, our results confirm the notion that Asp 116 is required for class I molecules to accommodate ligands with positively charged C-termini. The class I molecules known to present significant amounts of ligands terminated by a positively charged residue are HLA-A3 (Di Brino et al. 1993a; Maier et al. 1994), A11, A31, A33, Aw6801 (Guo et al. 1992), and B*2705 (Jardetzky et al. 1991). All contain two additional Asp residues inside or near the F-pocket, at positions 74 and 77. It appears that the lack of one Asp in any of these positions 74, 77, or 116 is correlated with a loss of the ability to present ligands with a basic C-terminus. HLA-A1 and B*2702 (Rötzschke et al. 1994), for example, have an Asn instead of an Asp at po-

sition 77, and both lack detectable amounts of such ligands. We obtained similar results with a mutant HLA-A*0201 molecule containing an Asp at 116 but a basic His at 74, where we could not find ligands with basic C-termini (O. Rötzschke and co-workers, unpublished). These results suggest that a strong negatively charged environment, provided by the three Asp residues at positions 74, 77, and 116, is required for efficient presentation of ligands with a positively charged C-terminus.

Acknowledgments Part of this work was carried out at the Max-Planck-Institut für Biologie, Tübingen. We thank Prof. J. Klein for support, Ms. Sachiko Karaki and Mr. S. Faath for technical assistance, Dr. P. Romero for CIR-A1 cells, and Ms. B. Stiller for preparing the manuscript. This study was supported by the Deutsche Forschungsgemeinschaft (SFB 120 and 323 and the Leibniz program), by a grant from the Bundesminister für Forschung und Technologie, and Grant-in-Aid for Scientific Research (B) (05454204), and for Scientific Research on Priority Areas (05272208) from the Ministry of Education, Culture, and Science, the Government of Japan.

References

- Alexander, J., Payne, J. A., Shigekawa, B., Frelinger, J. A., and Cresswell, P. The transport of class I major histocompatibility complex antigens is determined by sequences in the α_1 and α_2 protein domains. *Immunogenetics* 31: 169–178, 1990
- Barnstable, C. J., Bodmer, W. F., Brown, G., Galfre, G., Milstein, C., Williams, A. F., and Ziegler, A. Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens—new tools for genetic analysis. *Cell* 14: 9–20, 1978
- Bjorkman, P. J. and Parham, P. Structure, function, and diversity of class I major histocompatibility complex molecules. *Annu Rev Biochem* 59: 253–288, 1990
- Di Brino, M., Parker, K. C., Shiloach, J., Knierman, M., Lukszo, J., Turner, R. V., Biddison, W. E., and Coligan, J. E. Endogenous peptides bound to HLA-A3 possess a specific combination of anchor residues that permit identification of potential antigenic peptides. *Proc Natl Acad Sci USA* 90: 1508–1512, 1993a
- Di Brino, M., Tsuchida, T., Turner, R. V., Parker, K. C., Coligan, J. E., and Biddison, W. E. HLA-A1 and HLA-A3 T-cell epitopes derived from Influenza virus proteins predicted from peptide binding motifs. *J Immunol* 151: 5930–5935, 1993b
- Di Brino, M., Parker, K. C., Shiloach, J., Turner, R. V., Tsuchida, T., Garfield, M., Biddison, W. E., and Coligan, J. E. Endogenous peptides with distinct amino acid anchor residue motifs bind to HLA-A1 and HLA-B8. *J Immunol* 152: 620–631, 1994
- Falk, K., Rötzschke, O., Stevanović, S., Jung, G., and Rammensee, H.-G. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* 351: 290–296, 1991
- Falk, K., Rötzschke, O., Grahovac, B., Schendel, D., Stevanović, S., Jung, G., and Rammensee, H. G. Peptide motifs of HLA-B35 and HLA-B37 molecules. *Immunogenetics* 38: 161–162, 1993a
- Falk, K. and Rötzschke, O. Consensus motifs and peptide ligands of MHC class I molecules. *Sem Immunol* 5: 81–94, 1993
- Falk, K., Rötzschke, O., Grahovac, B., Schendel, D., Stevanović, S., Gnau, V., Jung, G., Strominger, J. L., and Rammensee, H.-G. Allele-specific peptide ligand motifs of HLA-C molecules. *Proc Natl Acad Sci USA* 90: 12005–12009, 1993b
- Gavioli, R., Kurilla, M. G., De Campos-Lima, P. O., Wallace, L. E., Dolcetti, R., Murray, R. J., Rickinson, A. B., and Masucci, M. G. Multiple HLA A11-restricted cytotoxic T-lymphocyte epitopes of different immunogenicities in the Epstein-Barr virus-encoded nuclear antigen 4. *J Virol* 67: 1572–1578, 1993
- Guo, H. C., Jardetzky, T. S., Garrett, T. P. J., Lane, W. S., Strominger, J. L., and Wiley, D. C. Different length peptides bind to HLA-Aw68 similarly at their ends but bulge out in the middle. *Nature* 360: 364–366, 1992
- Jardetzky, T. S., Lane, W. S., Robinson, R. A., Madden, D. R., and Wiley, D. C. Identification of self peptides bound to purified HLA-B27. *Nature* 353: 326–329, 1991
- Kato, N., Kikuchi, A., Kano, K., Egawa, K., and Takiguchi, M. Molecular analysis of a novel HLA-A33 subtype associated with HLA-B44. *Tissue Antigens* 41: 211–213, 1993
- Loveland, B., Wang, C. R., Yonekawa, H., Hermel, E., and Fischer Lindahl, K. Maternally transmitted histocompatibility antigen of mice: a hydrophobic peptide of a mitochondrially encoded protein. *Cell* 60: 971–980, 1990
- Madden, D. R., Gorga, J. C., Strominger, J. L., and Wiley, D. C. The structure of HLA-B27 reveals nonamer self-peptides bound in an extended conformation. *Nature* 353: 321–325, 1991
- Maier, R., Falk, K., Rötzschke, O., Maier, B., Gnau, V., Stevanović, S., Jung, G., Rammensee, H.-G., Meyerhans, A. Peptide motifs of HLA-A3, -A24, and B7 molecules as determined by pool sequencing. *Immunogenetics*, in press
- Malcherek, G., Falk, K., Rötzschke, O., Rammensee, H.-G., Stevanović, S., Gnau, V., Jung, G., and Melms, A. Natural peptide ligand motifs of two HLA molecules associated with myasthenia gravis. *Int Immunol* 5: 1229–1237, 1993
- Matsamura, M., Fremont, D. H., Peterson, P., and Wilson, I. A. Emerging principles for the recognition of peptide antigens by MHC class I molecules. *Science* 257: 927–934, 1992
- Missale, G., Redeker, A., Person, J., Fowler, P., Guilhot, S., Schlicht, H. J., Ferrari, C., and Chisari, F. V. HLA-A31- and HLA-Aw68-restricted cytotoxic T cell responses to a single hepatitis B virus nucleocapsid epitope during acute viral hepatitis. *J Exp Med* 177: 751–762, 1993
- Parham, P. and Bodmer, W. F. Monoclonal antibody to a human histocompatibility alloantigen, HLA-A2. *Nature* 276: 397–399, 1978
- Rötzschke, O., Falk, K., Stevanović, S., Gnau, V., Jung, G., and Rammensee, H.-G. Dominant aromatic/aliphatic C-terminal anchor in HLA-B*2702 and B*2705 peptide motifs. *Immunogenetics* 39: 74–77, 1994
- Saper, M. A., Bjorkman, P. J., and Wiley, D. C. Refined structure of the human histocompatibility antigen HLA-A2 at 2.6 Å resolution. *J Mol Biol* 219: 277–319, 1991
- Silver, M. L., Guo, H. C., Strominger, J. L., and Wiley, D. C. Atomic structure of a human MHC molecule presenting an influenza virus peptide. *Nature* 360: 367–369, 1992
- Sutton, J., Rowland-Jones, S., Rosenberg, W., Nixon, D., Gotch, F., Gao, X. M., Murray, N., Spoonas, A., Driscoll, P., Smith, M., Willis, A., and McMichael, A. A sequence pattern for peptides presented to cytotoxic T-lymphocytes by HLA-B8 revealed by analysis of epitopes and eluted peptides. *Eur J Immunol* 23: 447–453, 1993
- Udaka, K., Tsomides, T. J., Walden, P., Fukusen, N., and Eisen, H. N. A ubiquitous protein is the source of naturally occurring peptides that are recognized by a CD8⁺ T-cell clone. *Proc Natl Acad Sci USA* 90: 11272–11276, 1993
- Van der Bruggen, P., Traversari, C., Chomez, P., Lurquin, C., Deplaen, E., Vandeneuynde, B., Knuth, A., and Boon, T. A Gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 254: 1643–1647, 1991
- Zemmour, J., Little, A. M., Schendel, D. J., and Parham, P. The HLA-A,B negative mutant-cell line C1R expresses a novel HLA-B35 allele, which also has a point mutation in the translation initiation codon. *J Immunol* 148: 1941–1948, 1992
- Zhang, Q. J., Gavioli, R., Klein, G., and Masucci, M. G. An HLA-A11-specific motif in nonamer peptides derived from viral and cellular proteins. *Proc Natl Acad Sci USA* 90: 2217–2221, 1993

HLA-A31- and HLA-Aw68-restricted Cytotoxic T Cell Responses to a Single Hepatitis B Virus Nucleocapsid Epitope during Acute Viral Hepatitis

By Gabriele Missale,* Allan Redeker,† John Person,§
Patricia Fowler,* Stephane Guillhot,* Hans J. Schlicht,||
Carlo Ferrari,* and Francis V. Chisari*

From the *Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California 92037; the †Liver Unit, Rancho Los Amigos Medical Center, University of Southern California School of Medicine, Downey, California 90033; the §Department of Gastroenterology, Naval Hospital, San Diego, California 92134; and the ||Department of Virology, University of Ulm, 7900 Ulm, Germany

Summary

We have recently developed the technology to identify and characterize the human histocompatibility leukocyte antigen (HLA) class I-restricted, CD8⁺ cytotoxic T lymphocyte (CTL) response to hepatitis B virus (HBV)-encoded antigens in patients with acute viral hepatitis. CTL are expanded in vitro by stimulation with HBV-derived synthetic peptides and selected by restimulation with a panel of HLA-matched stable transfectants that express the corresponding HBV protein. We have recently reported the existence of an HLA-A2-restricted, CD8⁺ CTL response to an epitope located between residues 18 and 27 of the HBV nucleocapsid core antigen (HBcAg). We now report the discovery of a CTL epitope located between HBcAg residues 141 and 151 that completely overlaps a critical domain in the viral nucleocapsid protein that is essential for its nuclear localization and genome packaging functions as well as processing of the precore protein. The CTL response to this epitope is dually restricted by the HLA-A31 and HLA-Aw68 alleles, which, unexpectedly, appear to use a common binding motif based on the results of alanine substitution and competition analysis, and the binding properties of these two alleles predicted from their known primary sequence, and from the three-dimensional structure of HLA-Aw68. We have also demonstrated that the HBV-specific CTL response to this epitope is polyclonal during acute viral hepatitis, since these two restriction elements can present the HBcAg 141–151 epitope to independent CTL clones derived from a single patient; and that the CTL response is multispecific, since HLA-A2-restricted and HLA-Aw68-restricted CTL responses to HBcAg 18–27 and HBcAg 141–151, respectively, have been identified to coexist in another patient. The foregoing argue against the emergence of CTL escape mutants as a significant problem during HBV infection, especially at this locus, where mutations might be incompatible with viral replication. Finally, our data suggest an association between the HBV-specific CTL response and viral clearance, and they have implications for the design of immunotherapeutic strategies to terminate HBV infection in chronically infected patients.

The hepatitis B virus (HBV)¹ is a small hepatotropic enveloped virus with a double-stranded DNA genome (1) that causes acute and chronic necroinflammatory liver disease and hepatocellular carcinoma (2). The mechanisms responsible for viral clearance and liver cell injury in HBV infection are not well understood. Based on precedent in other viral

systems, and a limited analysis of the intrahepatic (3–5) and PBL (6) response to HBV-encoded antigens, it appears that HBV-specific helper and CTL responses may play an important pathogenetic role in this disease. Since the viral nucleocapsid protein is an important target of the CTL response to other viruses (7–11), we have begun to examine the HLA class I-restricted CTL response to the hepatitis B nucleocapsid core antigen (HBcAg) in patients with acute and chronic viral hepatitis, type B.

In a recent series of studies, we demonstrated that >90%

¹ Abbreviations used in this paper: HBcAg, hepatitis B core antigen; HBV, hepatitis B virus.

of HLA-A2-positive patients with acute HBV infection produce an HLA-A2-restricted, CD8-positive CTL response to a 10-residue epitope that maps between amino acids 18 and 27 (FLPSDFFPSV) of HBcAg (12). In contrast, we have shown that this response is not detectable in the peripheral blood in HLA-A2-positive patients with chronic hepatitis (13), suggesting that a vigorous CTL response to this epitope may contribute to viral clearance during HBV infection.

In the current study, we report the discovery and characterization of a new CTL epitope, recognized by patients with acute viral hepatitis, that maps to amino acids 141–151 of HBcAg (STLPETTVRR). CTL recognition of this epitope is restricted by two independent class I molecules, HLA-A31 and HLA-Aw68, and both responses are focused on precisely the same 11-residue sequence. To our knowledge this represents the first report that two independent human class I molecules can present precisely the same endogenously synthesized epitope to independently rearranged TCRs. Furthermore, our data provide insight into the probable antigenic peptide binding motifs for these two independent HLA alleles. Additionally, together with our previous reports (12–14), the current observations suggest that the HLA class I-restricted CTL response plays an important role in viral clearance during acute HBV infection, and they raise the possibility that specific augmentation of this response might lead to viral clearance in patients with chronic hepatitis.

Materials and Methods

Patient Population. Six patients, five male and one female, with acute hepatitis B and nine normal blood donors were studied (Table 1). The diagnosis of acute hepatitis B was based on standard diagnostic criteria including clinical and biochemical evidence of severe liver cell injury with alanine aminotransferase (ALT) activity at least 20-fold higher than the upper limits of normal, together with serological evidence of acute HBV infection, including hepatitis B surface antigen (HBsAg) and IgM anti-HBc antibody (IgM HBcAb), and the absence of serological evidence of infection by the hepatitis δ or hepatitis C viruses (using commercially available reagents obtained from Abbot Laboratories, North Chicago, IL). All patients recovered completely from the illness, with normalization of serum transaminase and clearance of HBsAg within 4 mo of initial diagnosis.

Synthetic Peptides, HBV Antigens, and Tetanus Toxoid. A panel of overlapping synthetic peptides, 10–20 residues long, corresponding to the complete HBV core protein (subtype ayw), were purchased from Multiple Peptide Systems (San Diego, CA) or provided by Cytel Corporation (San Diego, CA). Peptides representing amino- and carboxy-terminal truncations of HBcAg 140–155, and a panel of peptides containing alanine substitutions scanning HBcAg 141–151, were produced by Chiron Mimotopes (Clayton, Australia). Lyophilized peptides were reconstituted at 10 mg/ml in sterile distilled water with or without 10% DMSO (Malinckrodt, Paris, KY). Recombinant (r)HBcAg was obtained from bacterial extracts of *Escherichia coli* as previously described (15). Tetanus toxoid protein was purchased from Connaught Laboratories (Swiftwater, PA).

Recombinant Expression Vectors. Recombinant vaccinia viruses expressing the HBV core (c-vac) region (ayw subtype) and the control wild-type vaccinia have been previously described (16). An-

other construct (WTe) that produces the HBV precore protein (H. J. Schlicht, unpublished observations) was also used. Stable transfectants that express HBcAg (ayw subtype) were produced by transfection of EBV-transformed B lymphoblastoid cell lines (B-LCL) with a panel of EBV-based expressed vectors that contain the HBV core region as previously described (17).

Stimulation of PBMC with Synthetic Peptides and rHBc. PBMC from patients and normal donors were separated on Ficoll-Hypaque density gradients (Sigma Chemical Co., St. Louis, MO), washed three times in HBSS (Gibco Laboratories, Grand Island, NY), resuspended in RPMI 1640 (Gibco Laboratories) supplemented with L-glutamine (2 mM), penicillin (50 U/ml), streptomycin (50 μ g/ml), and Hepes (10 mM) containing 10% heat-inactivated human AB serum, and plated in a 24-well plate at 4×10^6 cells/well. The synthetic peptides, each at 10 μ g/ml, were added to the cell cultures as follows: mixture 1 (core residues 1–20, 20–34, 28–47, 50–69, 70–89, 61–80); mixture 2 (core residues 82–101, 100–119, 120–139, 140–155, 155–169, 169–183); mixture 3 (precore residue 20–core residue 2, core residues 50–59, 117–131, 131–145, 111–125); mixture 4 (core residues 11–27, 91–110, 147–160, 162–176). rHBcAg (Biogen, Geneva, Switzerland) was added at 1 μ g/ml to derive the benefit of a Th cell response within the culture during the first week of stimulation. At day 3, 1 ml of RPMI with 10% human AB serum and rIL-2 (Hoffman-La Roche, Inc., Nutley, NY) at 10 U/ml final concentration was added in each well, and the cultured PBMC were tested for CTL activity on day 7. PBMC that displayed cytolytic activity specific for one of the peptide mixtures used during the first week of stimulation were expanded by restimulation as described below.

Generation of HBV Core-specific CTL Lines. Generation of CTL line H1 from patient H.P., initially cultured with peptide mixture 2 plus rHBcAg, was performed by weekly restimulation with 5×10^5 autologous PBMC irradiated (3,000 rad) in RPMI plus 10% human AB serum, rIL-2 (20 U/ml), and peptide mixture 2 (first restimulation), or with peptide 140–155 (10 μ g/ml) for all subsequent stimulations. CTL line E4 (from patient E.W.) and the CTL lines from four additional patients (V.T., H.F., Q.M., C.N.) were established by stimulating the PBMC with peptide 140–155 plus rHBcAg for the first week, with weekly restimulation thereafter with peptide 140–155 and rIL-2. The PBMC of the normal uninfected controls were stimulated similarly except that in selected instances tetanus toxoid was substituted for rHBc during the first week of stimulation to provide an alternate source of T cell help, since these individuals had not been previously exposed to HBcAg.

Generation of HBV-specific CTL Clones. CTL clones were generated by limiting dilution at one cell/well in 96-well microtiter plates from an HBV-specific CTL line, E4 (patient E.W.). After depletion of CD4⁺ T cells from the CTL line by incubation with a CD4-specific mAb (Becton Dickinson & Co., Mountain View, CA) plus complement, the cells were plated in the presence of PHA (Sigma Chemical Co.) at 1 μ g/ml, CD3-specific mAb at 0.5 μ g/ml (Coulter Immunology, Hialeah, FL), rIL-2 (20 U/ml), and irradiated (5,000 rad) allogeneic PMBC (10^5 /well). HBV specific clones were restimulated in a 24-well plate with 10^5 irradiated (9,000 rad) autologous transfectants expressing the HBV core region (described above), with 2×10^6 allogeneic irradiated (3,000 rad) PBMC feeder cells per well, in RPMI 1640 containing 10% heat-inactivated FCS and IL-2 (20 U/ml).

Target Cell Lines. Autologous and allogeneic EBV-transformed B-LCL were either purchased from The American Society for Histocompatibility and Immunogenetics (A.S.H.I.; Boston, MA) or established from our own pool of patients and normal donors as described (18). Line LB, HLA-Aw68.1⁺ (described in reference

19), provided by Dr. D. C. Wiley, and line RSH (described in reference 19, purchased from A.S.H.I., were used in selected experiments. The cells were maintained in RPMI with 10% (vol/vol) heat-inactivated FCS (Gibco Laboratories). Short-term lines of autologous PBMC blasts were produced by stimulating PBMC with PHA at 1 μ g/ml in RPMI with 10% FCS, 10 U/ml rIL-2 for 7 d before use as target cells (see below).

Cytotoxicity Assay. Target cells consisted either of: (a) autologous PHA-stimulated blasts or allogeneic HLA-matched and -mismatched B-LCL incubated overnight with synthetic peptides at varying concentrations between 0.001 and 10 μ g/ml; (b) stable B-LCL transfectants described above; or (c) B-LCL infected with recombinant vaccinia viruses. In selected experiments (see Results) the target cells were labeled with 51 Cr for 1 h, followed by 1 h of incubation with synthetic peptides before the addition of effector cells. Vaccinia-infected targets were prepared by infection of 10^6 cells at 50 PFU/cell on a rocking plate at room temperature for 1 h followed by a single wash and overnight incubation at 37°C. Target cells were then labeled with 100 μ Ci of 51 Cr (Amersham Corp., Arlington Heights, IL) for 1 h and washed three times with HBSS. Cytolytic activity was determined in a standard 4-h 51 Cr release assay using U-bottomed 96-well plates containing 5,000 targets/well. Stimulated PBMC from patients and normal controls were tested at E:T ratios between 60 and 100:1, whereas HBV core-specific CTL lines were tested at E:T ratios between 4 and 50:1. All assays were performed in duplicate. Percent cytotoxicity was determined from the formula: $100 \times [(\text{experimental release} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})]$. Maximum release was determined by lysis of targets by detergent (1% Triton X-100; Sigma Chemical Co.). Spontaneous release was <25% of maximal release in all assays.

Flow Cytometry Analysis. Cells to be analyzed ($0.5\text{--}1.0 \times 10^6$ cells) were washed once in PBS and then incubated with a fluorescent probe-conjugated anti-CD4 and anti-CD8 mAb (Leu3a, Leu2a) and similarly labeled control antibody (Becton Dickinson & Co.). After a 30-min incubation at 4°C, cells were washed in PBS with 5% BSA and 0.1% sodium azide, and analyzed with a FACScan® flow cytometer (Becton Dickinson & Co.).

HLA Typing. HLA typing of PBMC from patients and from normal donors was performed by microcytotoxicity using HLA typing trays purchased from One Lambda (Los Angeles, CA). The HLA haplotypes of six patients and nine uninfected normal controls used in this study are shown in Table 1.

Results

CTL Activity of PBMC Stimulated with Peptide Mixtures. PBMC from two patients (E.W., H.P.) with acute HBV infection were stimulated for 7 d with peptide mixtures, and then tested for cytolytic activity against autologous 51 Cr-labeled, PHA-activated blasts prepulsed with the same peptide mixture or with media. Interestingly, responses were observed to mixture 2 in both patients (Fig. 1). Synthetic peptides contained in mixture 2 represent HBcAg sequences that have not been previously described as recognition sites for HBV-specific human CTL. Accordingly, the remaining cells were restimulated for a second week with peptide mixture 2, and the antigenic specificity of the restimulated CTL line was established with autologous PHA-activated blast targets prepulsed with the individual peptides contained within the mixture. By this approach, peptide HBcAg 140–155 was

Table 1. HLA Class I Haplotype of the HBV-infected Patients, and the HLA-A31- and Aw68-positive Normal Donors Included in This Study

Patient	HLA class I
HBV patient	
E.W.	A31, Aw68, B35, Cw3, Cw4
H.P.	A2, Aw68, B35, Bw62, Cw3, Cw4
V.T.	A25, A31, B7, B18
H.F.	A31, Aw68, Bw61, Cw3
Q.M.	Aw36, Aw68, B49, Bw62, Cw1
V.P.	A24, Aw68, B35, Bw67
C.N.	A24, Aw68, Bw60, Cw3
Normal donor	
a	A1, A31, B17, Bw60
b	A2, A31, B27, B44, Cw1
c	A3, A31, B7, B27
d	A24, A31, B14, B35, Cw4
e	A3, A31, B7
f	A31, Aw68, B35, Bw60
g	A3, Aw68, B7, B44, Cw7
h	A1, Aw68, B8, B38, Cw7
i	A11, Aw68, B35, B44, Cw4

shown to be responsible for the CTL activity induced by mixture 2 for both of these patients (Fig. 2). No cytotoxic activity was observed using unstimulated PBMC of patient E.W. as effectors against autologous B-LCL fed with peptide HBcAg 140–155 (data not shown), suggesting that specific CTL are present at low frequency in the peripheral blood during acute HBV infection. It is noteworthy that patient H.P. also displayed a CTL response to peptide mixture 4, and that pa-

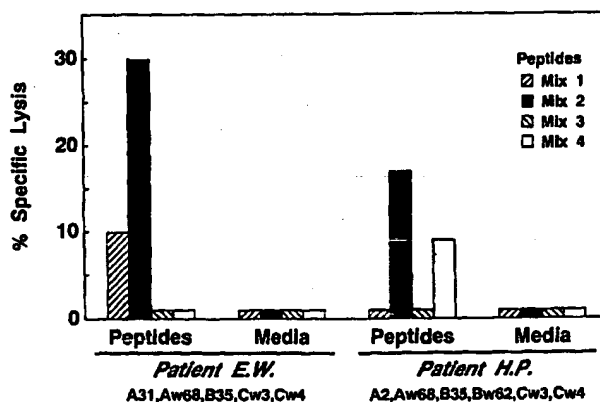


Figure 1. Activation of HBV-specific CTL by PBMC stimulation with mixtures of synthetic peptides. PBMC were stimulated for 1 wk with mixtures of HBcAg peptides and tested for cytotoxic activity in a 4-h 51 Cr release assay against autologous targets prepulsed with the same stimulatory mixture or with media only. The E/T ratio used was 70:1 for patient E.W. and 100:1 for patient H.P.

tient E.W. displayed a CTL response to mixture 1 (Fig. 1). Although the response to mixture 1 was transient and could not be further characterized, the response to mixture 4 was ultimately shown to be specific for core residues 18–27 and to be restricted by HLA-A2 (not shown), as we have previously reported (12–14, 17), demonstrating that multiple, independently restricted CTL responses to nonoverlapping CTL epitopes present on the same viral protein are readily detectable during acute HBV infection.

Generation and HLA Restriction Analysis of HBcAg 140–155-specific CTL Lines and Clones. HBcAg 140–155-specific CTL lines were generated by weekly stimulation of PBMC either with mixture 2 or with the active constituent peptide (core residues 140–155). Line E4 (patient E.W.) was started in the presence of rHBcAg and peptide HBcAg 140–155; line H1 (patient H.P.) was started in the presence of rHBc and mixture 2.

After 4 wk of restimulation, the HLA class I restriction of CTL line H1 was tested by using several allogeneic B-LCL target cells that were partially matched with the effector cells at the HLA class I loci but were completely HLA class II mismatched. The results, shown in Fig. 3, illustrate that the CTL activity was HLA-Aw68 restricted. The HBcAg 140–155-specific CTL line E4 was cloned at one cell per well in the

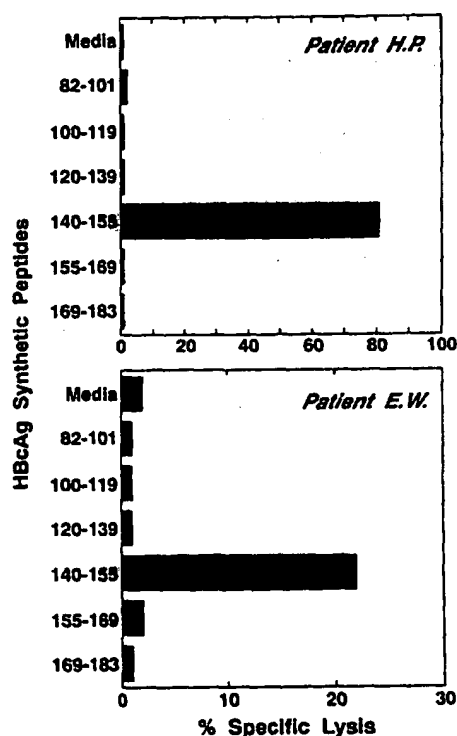


Figure 2. Identification of the target peptide in mixture 2. PBMC, stimulated by peptide mixture 2 for the first week, were restimulated with the same mixture of peptides and tested for cytotoxic activity in a 4-h ^{51}Cr release assay against ^{51}Cr -labeled autologous target cells prepulsed with the individual peptides in the mixture. The E/T ratio used was 50:1 for patient E.W. and 40:1 for patient H.P.

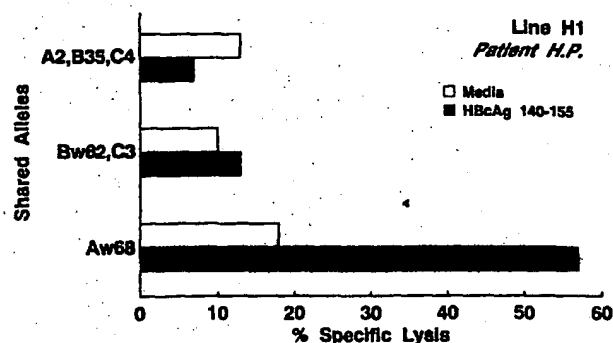


Figure 3. HLA restriction of the HBcAg 140–155-specific CTL response in patient H.P. A polyclonal CTL line (H1) specific for HBcAg 140–155 was incubated with ^{51}Cr -labeled B-LCL target cells that were prepulsed with peptide HBcAg 140–155 or with media. All target cells were completely mismatched at the level of class II and partially matched at the level of class I. The E/T ratio used was 4:1 in a 4-h ^{51}Cr release assay.

presence of anti-CD3, PHA, and allogeneic PBL as feeder cells. After 2–3 wk, 15% of the seeded wells showed proliferation, and the growing cell populations were tested for specific lysis of autologous B-LCL preincubated with peptide core 140–155. Two clones (3D11, 2D7) that displayed highly efficient specific cytotoxic activity were selected for further analysis. The clones were tested against autologous and allogeneic B-LCL target cells partially matched with the effectors at the level of HLA class I and II alleles. The cytolytic activity of clone 3D11 was found to be HLA-A31 restricted, and the cytolytic activity of clone 2D7, derived from the same patient, was HLA-Aw68 restricted (Fig. 4). Both clones displayed the CD4^- , CD8^+ phenotype by flow cytometry (not shown).

These observations were confirmed and extended by analysis of four additional HLA-A31- or Aw68-positive patients

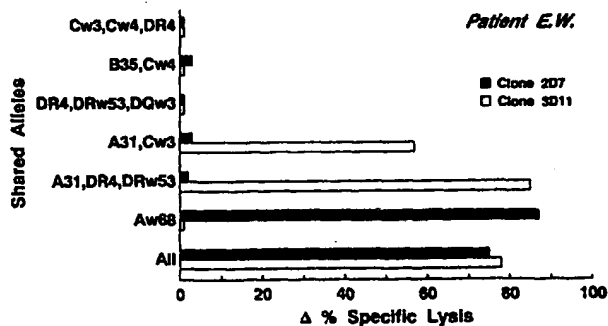


Figure 4. HLA restriction of the HBcAg 140–155-specific CTL response in patient E.W. Two HBcAg 140–155-specific CTL clones (3D11 and 2D7) were established by limiting dilution and incubated with ^{51}Cr -labeled autologous or allogeneic B-LCL target cells prepulsed with peptide 140–155 or media. Allogeneic target cells were matched at the level of class I and class II with the effector cells. The E/T ratio used was 10:1 in a 4-h ^{51}Cr release assay. Results are expressed as the difference between the percent ^{51}Cr release observed with peptide-pulsed targets and the percent ^{51}Cr release with media-pulsed target cells (which was <3% in all cases).

with acute HBV infection (H.F., V.T., Q.M., C.N.). In all these patients, HBcAg 140-155-specific CTL lines were generated as described for line E4. Using partially HLA-matched allogeneic target cells, the CTL response was shown to be restricted by the HLA-A31 allele in patient V.T.; it was clearly HLA-Aw68 restricted in patient Q.M. and probably Aw68 restricted in patient C.N. (Table 2). The response in patient H.F. was too weak to permit further analysis.

HBcAg 140-155-specific CTL Lines and Clones Can Lyse Target Cells That Express Endogenously Synthesized HBcAg. Two polyclonal CTL lines (E4, H1) and two clones derived from line E4 (3D11, 2D7) were tested for the ability to recognize endogenously synthesized nucleocapsid antigen by using autologous and allogeneic target cells that had been infected with recombinant vaccinia viruses that direct the synthesis of the HBV core and precore proteins by the cell. Line H1 (patient H.P.) and line E4 (patient E.W.) were tested against endogenously synthesized core protein induced by a recombinant vaccinia virus designated core-vac (Fig. 5). Clones 3D11 and 2D7 were tested against endogenously synthesized core and precore proteins induced by a recombinant vaccinia virus, designated core-vac and WT-vac, respectively (Fig. 6). Significant levels of specific cytolytic activity were detected in all cases (Figs. 5 and 6). Recognition of endogenously synthesized antigen by HBcAg 140-155 peptide-specific lines and clones demonstrates that the CTL epitope represented by the core sequence 140-155 is actually generated by the intracellular processing of endogenously synthesized HBV core and precore proteins, and that these CTL are likely primed *in vivo* during HBV infection. The latter argument is confirmed by our repeated failure to establish HBcAg 140-155-specific CTL lines from six HLA-A31-positive or from four HLA-Aw68-positive normal uninfected controls that we have studied thus far (not shown).

The 11-mer HBcAg 141-151 Is the Minimum, Optimally Recognized HLA A31- and Aw68-restricted Epitope within HBcAg 140-155. Carboxy- and amino-terminal truncations of the core 140-155 sequence were produced to map the CTL epitopes that are restricted by HLA-A31 and Aw68 within this 16-residue peptide (Table 3). Clone 3D11, which is HLA A31

Table 2. HBcAg 140-155-specific Cytotoxicity of CTL Lines from HLA-A31- and Aw68-positive Patients with Acute HBV Infection

Patient	HLA match	Target	
		HBcAg 140-155	Media
V.T.	A31	75	34
H.F.	All	10	1
Q.M.	Aw68	23	0
C.N.	Aw68,A24	25	10

PBMC stimulated with HBcAg 140-155 plus rHBcAg. Data represent percent ^{51}Cr release.

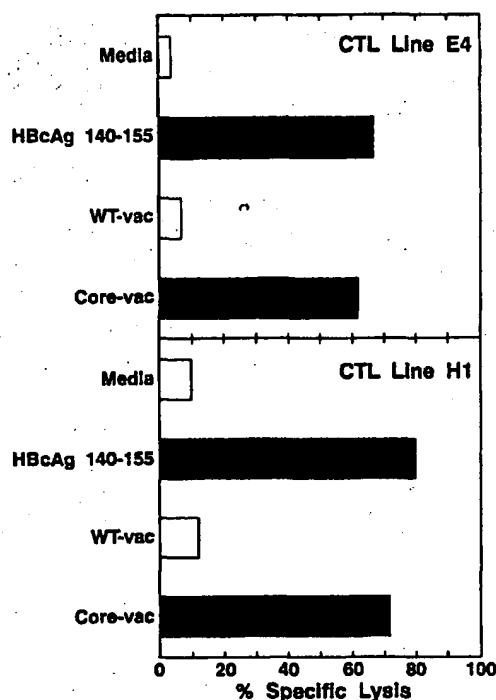


Figure 5. Recognition of endogenously synthesized HBcAg by HBcAg 140-155-specific CTL lines E4 (HLA-A31 and Aw68 restricted) and H1 (HLA-Aw68 restricted). CTL lines were incubated for 4 h with ^{51}Cr -labeled autologous B-LCL (line E4) and allogeneic B-LCL (line H1) matched at HLA-Aw68, which had been prepulsed with media or HBcAg 140-155 peptide, or were infected with recombinant vaccinia viruses that express the HBV core protein. The E/T ratio used for line E4 was 10:1, for line H1 was 20:1 for the peptide prepulsed target cells, and was 15:1 for the recombinant vaccinia-infected target cells.

restricted, and clone 2D7, which is HLA Aw68 restricted, were used to define the fine specificity of the CTL response. Autologous B-LCL were preincubated with the truncated peptides (10 μM) and used as targets with the two clones. We

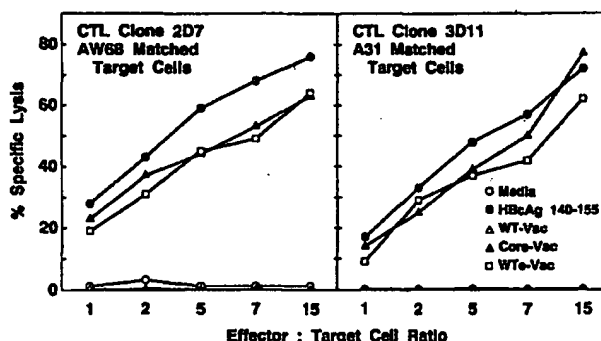


Figure 6. HLA-restricted recognition of endogenously synthesized HBcAg by CTL clones 2D7 and 3D11. Clones 2D7 and 3D11 were tested against HLA-Aw68- and A31-matched allogeneic target cells, respectively. Targets were either preincubated with peptide HBcAg 140-155, or infected with recombinant vaccinia viruses coding for the core (Core-Vac) or the precore (WT-Vac) proteins of HBV in a 4-h ^{51}Cr release assay.

Table 3. *Fine Specificity of the Cytotoxic Activity of Clones 3D11 and 2D7*

Peptides		3D11	2D7
Residues	Sequence	A31	Aw68
140-155	LSTLPETTIVRRRGRS	72	62
140-154	LSTLPETTIVRRRGR	63	60
140-153	LSTLPETTIVRRRG	75	66
140-152	LSTLPETTIVRRR	77	69
140-151	LSTLPETTIVRR	72	67
140-150	LSTLPETTIVR	0	6
141-155	STLPETTIVRRRGRS	81	66
141-154	STLPETTIVRRRGR	79	67
141-153	STLPETTIVRRRG	79	59
141-152	STLPETTIVRRR	68	68
141-151	STLPETTIVRR	69	66
141-150	STLPETTIVR	20	52
141-149	STLPETTIV	0	3
142-155	TLPETTIVRRRGRS	8	63
142-154	TLPETTIVRRRGR	18	54
142-153	TLPETTIVRRRG	8	56
142-152	TLPETTIVRRR	2	37
142-151	TLPETTIVRR	47	60
142-150	TLPETTIVR	0	0
143-155	LPETTIVRRRGRS	0	0
143-154	LPETTIVRRRGR	0	0
143-153	LPETTIVRRRG	0	0
143-152	LPETTIVRRR	0	2
143-151	LPETTIVRR	0	0

Autologous target cells were preincubated with peptides at 10 μ M; E/T ratio, 10:1. Lysis was of target cells preincubated with media <2% in all cases. Data represent percent ^{51}Cr release.

expected to identify two partially overlapping epitopes in peptide HBcAg 140-155. Surprisingly, however, our data indicate that sequence 141-151 is the minimal, optimally recognized epitope for both restriction elements. Initial studies using the truncated peptides at a single concentration (Table 3) defined residue 151 (arginine) as the putative carboxy terminus of the epitope recognized by both CTL clones. Interestingly, residue 150, which is also an arginine, can also serve as the carboxy-terminal residue for both clones but only if residue 141 (serine) serves as amino terminus. Although serine 141 appears to be the optimal amino-terminal residue of the epitope for both restriction elements, residue 142 (threonine) can also serve as the amino terminus of the epitope for both clones if arginine 151 is the carboxy-terminal residue. In contrast, only the HLA-Aw68-restricted clone (2D7) can use threonine 142 if the carboxy terminus of the peptide is extended beyond residue 151. The chemical similarity of the two amino-terminal residues (serine 141, threonine 142) and the identity

of the two carboxy-terminal residues (arginine 150, 151) probably contribute to these observations.

To precisely define the boundaries of the epitope(s), a dose titration analysis was conducted in which the two CTL clones were incubated with allogeneic HLA-A31- and HLA-Aw68-positive target cells preincubated with peptides 140-155, 140-153, 140-151, 141-151, 141-150, 141-152, and 142-151 at different molar concentrations ranging from 10^{-3} to 10 μ M. From these studies it is clear that core residues 141-151 represent the minimal optimally recognized epitope recognized by both of the CTL clones (Fig. 7), suggesting that both HLA alleles bind and present exactly the same peptide to their corresponding CTL. The availability of HLA-Aw68.1- and HLA-Aw68.2-positive cell lines permitted us to define the HLA-Aw68 subtype responsible for presentation of HBcAg 141-151 to clone 2D7. HLA Aw68.1-positive target cells pulsed with HBcAg 141-151 (1 μ M) were efficiently lysed (39% specific ^{51}Cr release) by clone 2D7 at an E/T ratio of 10:1,

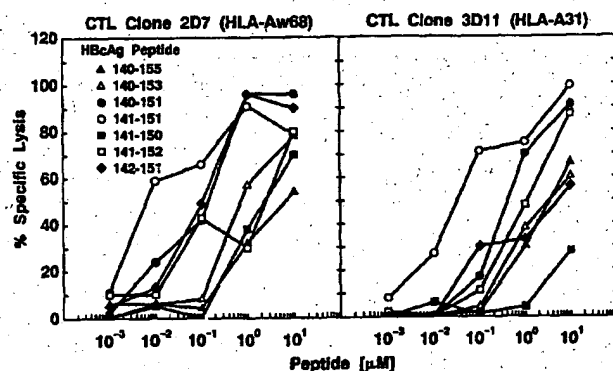


Figure 7. Identification of the shortest optimally recognized sequence in HBcAg 140–155 for each HLA restriction element. Clone 3D11 was tested against allogeneic ^{51}Cr -labeled A31-positive target cells and clone 2D7 against allogeneic ^{51}Cr -labeled Aw68-positive target cells prepulsed for 1 h with various peptides at concentrations ranging between 0.001 and 10 μM . Target cells were incubated in presence of peptides at 37°C for 1 h and subsequently diluted 20-fold in RPMI 1640 containing 10% FCS and added to the effector cells for 4 h at an E/T ratio of 10:1.

in contrast to HLA-Aw68.2-positive targets, which displayed no specific lysis under the same conditions (not shown).

Identification of Critical Residues Involved in Presentation and Recognition of HBcAg 141–151. To define the residues within HBcAg 141–151 that interact with the HLA-A31 and Aw68 molecules and that are recognized by the CTL receptors of clones 3D11 and 2D7, respectively, a series of peptides containing individual alanine substitutions at each residue (A141–A151) were produced. Allogeneic HLA-A31- and HLA-Aw68-matched target cells were pulsed with each peptide at multiple concentrations between 0.001 and 10 μM and used in a 4-h ^{51}Cr release assay with clones 3D11 and 2D7 at an E/T ratio of 10:1. Comparison of the dose-response curves for each substituted peptide revealed a hierarchy that is illustrated in Fig. 8, with the results obtained at a single peptide concentration (1 μM) for clarity. As shown in Fig. 8, the two clones displayed similar, but not identical, peptide recognition profiles. Interestingly, substitutions of leucine 143 and arginine 151 either reduced or nearly abolished CTL-mediated killing by both clones. In contrast to these shared substitutions, threonine 146 and threonine 147 substitutions almost completely abolished CTL-mediated killing by clone 2D7, but they had no effect on clone 3D11. On the contrary, substitution of glutamic acid 145, valine 149, and arginine 150 almost completely abolished recognition by 3D11 but had no effect on 2D7. Although it is clear that these residues are involved in peptide interaction with either the HLA class I restriction elements or with the TCRs of the corresponding CTL clones, the fact that leucine 143 and arginine 151 appear to be important for both HLA-A31- and the HLA-Aw68-restricted responses suggests to us that these residues may mediate the binding of HBcAg 141–151 to both HLA alleles (agretope), while threonine 146 and 147 may represent residues that interact with the TCR of clone 2D7 (epitope), whereas glutamic acid 145, valine 149, and arginine 150 might contribute to the epitope recognized by the 3D11 TCR.

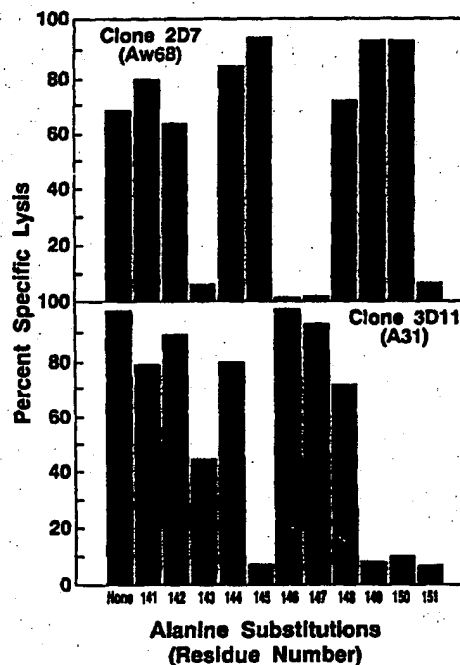


Figure 8. Recognition of HBcAg 141–151 peptides containing alanine substitutions at each residue. Identification of critical residues involved in presentation and recognition of HBcAg 141–151. Clone 3D11 was tested against ^{51}Cr -labeled allogeneic A31-positive target cells, and clone 2D7 against allogeneic ^{51}Cr -labeled Aw68-positive target cells that had been prepulsed for 1 h with varying concentrations (0.001–10 μM) of HBcAg 141–151 or with a panel of modified peptides in which alanine was substituted at each position. Target cells were incubated in presence of peptides at 37°C for 1 h and subsequently diluted 20-fold in RPMI 1640 containing 10% FCS and added to the effector cells for 4 h at an E/T ratio of 8:1. Representative results obtained at 1 μM peptide concentration are shown.

To distinguish between HBcAg 141–151 residues that interact with the HLA-A31 and Aw68 molecules and those residues that are seen by the CTL receptor in clones 3D11 and 2D7, we examined the ability of alanine-substituted peptides (100 μM) to prevent the binding of the native HBcAg 141–151 peptide (0.05 and 0.01 μM , respectively) to HLA-A31- and HLA-Aw68-positive target cells (Fig. 9).

Our results demonstrate that alanine substitutions of glutamic acid 145 and valine 149 effectively block CTL recognition of HBcAg 141–151 by HLA-A31-restricted clone 3D11, indicating that they probably represent TCR binding residues (epitope). In contrast, peptides containing alanine substitutions at residues leucine 143 and arginine 150 and 151 do not block the binding of HBcAg 141–151 to the HLA-A31 molecule, suggesting that some or all of these three residues are probably involved in the interaction of HBcAg 141–151 with HLA-A31. The results are particularly convincing for positions 150 and 151 because the corresponding alanine-substituted peptides do not sensitize the target cells to lysis by clone 3D11 at the high concentrations used for competition in this experiment. However, because the alanine 143-substituted peptide itself was recognized at the high con-

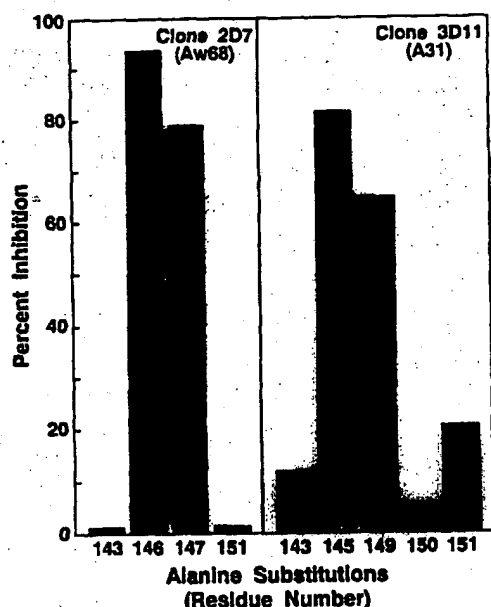


Figure 9. Competitive inhibition binding of HBcAg 141–151 to HLA-Aw68 and A31. Identification of residues within HBcAg 141–151 responsible for interaction with HLA-A31 and HLA-Aw68, and with the CTL receptors in clones 2D7 and 3D11. Peptides containing alanine substitutions at the indicated positions (100 μ M) were added to HLA-Aw68- or HLA-A31-positive target cells for 10 min, after which HBcAg 141–151 was added as a target peptide at 0.01 or 0.05 μ M, respectively. 1 h later the target cells were diluted 20-fold in complete media and the corresponding CTL clones were added at an E/T ratio of 10:1 in a 4-h 51 Cr release assay. Results are expressed as percent inhibition of the HBcAg 141–151-specific lysis observed in the absence of competitor peptide.

centrations required for this experiment, additional experiments were performed using a peptide with a nonconservative substitution (glutamic acid) at position 143. This peptide did not sensitize target cells even at high concentrations and it did not compete with the unsubstituted HBcAg 141–151 when it was used at 100-fold molar excess. Thus, the data suggest that leucine 143 and arginine 150 and 151 are probably HLA-A31 contact residues.

For the HLA-Aw68-restricted CTL clone 2D7, alanine substitution of threonine 146 and threonine 147 effectively block CTL recognition of HBcAg 141–151, indicating that these residues probably represent a TCR residue (epitope). On the other hand, peptides containing alanine substitutions at residues leucine 143 and arginine 151 do not block the binding of HBcAg 141–151 to the HLA-Aw68 molecule, even at 10,000-fold molar excess (concentrations that are entirely non-sensitizing for lysis by clone 2D7). These data strongly suggest that both of these residues are necessary for interaction of HBcAg 141–151 with HLA-Aw68.

Discussion

In the present study we have identified a new CTL epitope in the nucleocapsid protein of the HBV, corresponding to HBcAg residues 141–151, by synthetic peptide stimulation

of PBL from patients with acute viral hepatitis. We have shown that this epitope is generated by the cellular processing of endogenously synthesized HBV core and precore proteins, and that the CTL response to this single 11-residue minimal epitope is restricted by two different HLA molecules, HLA-A31 and HLA-Aw68. Finally, we have defined, at least partially, the agretopic and epitopic residues in HBcAg 141–151 that are seen by two independently restricted CTL clones, and by so doing we have tentatively identified a common, allele-specific binding motif for HLA-A31 and Aw68.

As we have reported previously (12–14, 17), we used synthetic peptides to expand the quantitatively limited, peripheral blood CD8-positive HBV antigen-specific cytotoxic T cell population in our patients. This strategy is necessary because HBV does not readily infect human cell lines *in vitro* and because our panel of stable HBV transfectants (17) apparently do not express sufficient amounts of endogenously processed antigen on the cell surface for efficient *in vitro* secondary induction of an HBV antigen-specific, class I-restricted peripheral blood CTL response in the HBV-infected patients we have studied thus far. In contrast, CTL stimulation with synthetic peptides is a powerful strategy to expand antigen-specific CTL populations that have been primed *in vivo* since we have shown, here and elsewhere (14, 17), that the peptide-stimulated CTL can efficiently recognize targets that express endogenous antigen. It is interesting that the stable transfectants can be very effectively used to select, expand, and maintain CTL that preferentially recognize endogenously synthesized antigen from the peptide-stimulated CTL population despite their inability to expand the same antigen-specific CTL without prior stimulation by the synthetic peptides. This may reflect the requirement for a higher epitope density for induction of a CTL response than for its maintenance, as previously described (14, 17). Nonetheless, the use of these transfectants for this purpose is important because it selects for the expansion of CTL populations that recognize endogenously synthesized epitopes rather than those subpopulations of CTL that only recognize the peptide used for the initial expansion.

It is noteworthy that the current stimulation strategy has permitted us to detect a CTL response to two independent epitopes in patient H.P., who displayed a response to the HLA-A2-restricted HBcAg 18–27 epitope that we have previously reported (12–14, 17) and to the HLA-Aw68-restricted epitope HBcAg 141–151 reported herein. Furthermore, patient E.W. yielded two independent CTL clones whose antigenic fine specificity differed according to the HLA allele that restricted the response (glutamic acid 145 and valine 149 for the HLA-A31-restricted clone, and the two threonine residues at positions 146 and 147 for the clone restricted by HLA-Aw68). These data, and others not presented in this study (F. V. Chisari et al., unpublished observations), suggest that the CTL response to HBV in humans is both polyvalent and multispecific, presumably to afford efficient protection from this serious viral infection.

Another interesting result is the efficient CTL recognition of target cells expressing endogenously synthesized core

and precore proteins (Fig. 6), which are identical except for the presence of a 29-residue amino-terminal signal sequence in the precore protein that directs it to the endoplasmic reticulum where 19 amino-terminal residues and 34 carboxy-terminal residues (residues 149–183) are removed and the product is secreted via the Golgi apparatus into the serum as HBcAg. In contrast, the core protein lacks the signal sequence and is targeted principally to the nucleus by a series of arginine-rich localization signals at its carboxy terminus (20); one of which (residues 145–156) also plays a critical role in viral RNA encapsidation (21). It is interesting that residues 141–151 are involved in all of these important events in the virus life cycle. The significance of this observation is not known at present. However, the CTL response to this epitope could be especially effective at viral clearance because in addition to destroying HBV-infected cells, CTL-resistant “escape” mutants at this epitope would probably be nonviable. The current data indicate that, despite their different intracellular trafficking pathways, the core and precore proteins appear to intersect either at the level of proteolytic processing in the cytoplasm or at the level of MHC binding within the endoplasmic reticulum since target cells that synthesize either protein are equally recognized by HBcAg 18–27 (14) and HBcAg 141–151-specific CTL (Fig. 6).

Relatively few antigenic determinants have been described that are broadly presented by multiple MHC molecules. In most instances the responses have been class II restricted (22, 23), although a murine CTL epitope in HIV-1 envelope glycoprotein has been recently described that can be presented by at least four different class I MHC molecules from independent haplotypes (24). However, at the human class I-restricted CTL level, broadly restricted responses have been described only for partially overlapping epitopes (25), or for antigens in which the minimal CTL epitope has not been precisely determined (26). To our knowledge, therefore, this is the first report that a single, precisely defined CTL epitope generated by the intracellular processing of an endogenously synthesized protein effectively induces a specific CTL response that is restricted by two independent HLA class I alleles *in vivo* in humans.

Insight into the molecular basis for this event can be gained by comparing the structural and chemical properties of the antigen-binding pockets in the HLA molecule with the results of our alanine substitution experiments. The polymorphic HLA class I protein is characterized by 20 highly variable residues, 16 of which are thought to contribute to formation of at least two pockets that have been identified in the antigen binding groove of the HLA-A2 and HLA-Aw68 molecules (27–30). These pockets are presumed to represent the binding sites of critical anchor residues within antigen peptides that mediate their binding to the HLA class I molecule.

Structural analysis reveals that one of these pockets is formed by hydrophobic residues at positions 24, 26, 34, and 67 of the class I heavy chain plus a highly variable residue at position 45 (30). Comparative DNA sequence analysis reveals that this pocket should be present in all HLA-A molecules, except perhaps HLA-A1 (31). The existence of a hydrophobic pocket

in the HLA-A2 protein is compatible with recent observations that leucine and other hydrophobic amino acids located at position 2 represent a critical anchor residue for antigenic nonamer and decamer peptides that can be eluted from the HLA-A2.1 binding groove (32, 33). It is also consistent with our previous identification of leucine at position 2 in a 10-amino acid HBcAg-derived sequence (HBcAg 18–27) (FLPSDFFPSV) that is a major, HLA-A2-restricted CTL epitopes in patients with acute viral hepatitis (12).

It is important to note that leucine 143 is present as residue 3 of the HLA-A31- and HLA-Aw68-restricted CTL epitope described in the current report (STLPETTVVRR). This observation raises the possibility that leucine 143 may contribute, as an anchor residue, to the binding of this peptide to both restriction elements. Indeed, our experimental results (Figs. 8 and 9) suggest that this is true although the relative importance of this residue in binding to the HLA-A31 and Aw68 molecules may be somewhat different.

Structural analysis of the HLA-Aw68 protein reveals the presence of a prominent negatively charged pocket, defined by an aspartic acid in position 74 in both HLA-Aw68.1 and 68.2 subtypes, plus two residues at positions 97 and 116, which differ between the two subtypes (Aw-68.1, methionine and aspartic; Aw68.2, arginine and tyrosine). Because of these differences, positively charged residues like arginine, in antigenic peptides, have access to the 74 pocket in HLA-Aw68.1 but not in Aw68.2 (31). Comparative DNA sequence analysis reveals that residues 74, 97, and 116 are all conserved in HLA-Aw68, A29, A31, A32, and A33 molecules but not in other class I alleles.

Since a positively charged carboxy-terminal residue (arginine 151) is required for recognition of the current epitope by both HLA-Aw68- and A31-restricted CTL (Table 3 and Figs. 7 and 8), it is possible that it represents a second important anchor residue required for peptide interaction with both of these HLA molecules. This is supported by the alanine substitution studies, which demonstrate that arginine 151 is critical for presentation of HBcAg 141–151 to both CTL clones (Fig. 8) but that it does not behave as an epitopic residue in blocking studies illustrated in Fig. 9, suggesting that it is probably involved in interaction with the HLA class I molecules. The data also suggest that arginine 150 may bind to HLA-A31 but not to Aw68 (Figs. 8 and 9). It is pertinent to point out, at this point, that an arginine has also been suggested to be the anchor residue for the 74 pocket of HLA-Aw68 for an influenza CTL epitope (19). Furthermore, the inability of HLA-Aw68.2-positive target cells to present HBcAg 141–151 to clone 2D7, while HLA-Aw68.1-positive target cells are fully functional, supports our belief that the carboxy-terminal arginine anchors HBcAg 141–151 to the 74 pocket of the HLA-Aw68.1 molecule.

As mentioned above, our data suggest that the epitopic residues within HBcAg 141–151 that interact with the CTL receptor are different for the two clones we have studied. The HLA-A31-restricted CTL clone 3D11 recognizes glutamic acid 145 and valine 149 while the HLA-Aw68-restricted CTL clone 2D7 recognizes the two threonine residues at positions 146

and 147. It will be interesting to generate additional HLA-A31- and Aw68-restricted, HBcAg-specific CTL clones in the future to assess the degree to which the CTL repertoire varies with respect to recognition of this HBV-encoded antigen.

Finally, the identification of this new CTL epitope in the HBV nucleocapsid protein is relevant to HBV immunobiology and pathogenesis for several reasons.

First, it expands our knowledge of the HBV-specific CTL repertoire to a new domain within the viral nucleocapsid protein and to two new HLA class I restriction elements.

Second, it demonstrates, for the first time, that the HBV-specific CTL response during acute viral hepatitis extends to multiple viral epitopes and different restriction elements; i.e., it is polyclonal, multispecific, and polymorphically restricted, thereby affording enhanced protection for the host against a pathogen that is as dangerous and as prone to mutate as HBV.

Third, it demonstrates that the HBV core and precore proteins are equally good targets of a nucleocapsid-specific CTL response to a shared epitope such as HBcAg 141-151, as we have previously demonstrated for HBcAg 18-27 (14). This implies that viruses containing mutations that affect only one of these proteins, such as the well-described precore stop codon mutation that blocks precore protein translation without affecting the core protein (34, 35), probably do not represent CTL escape mutants, since cells infected by the mutant viruses should still produce the HBcAg 141-151 epitope and be elim-

inated by the CTL. This further implies that factors other than CTL-imposed selection pressure are probably responsible for emergence of the precore codon 28 mutants that appear to be more virulent than wild-type forms of HBV (35).

Fourth, it focuses our attention on a functionally important domain within the HBV nucleocapsid region that is involved not only in nuclear localization of HBcAg (20) but also in viral RNA encapsidation (21), and also in cleavage of the precore protein to produce HBeAg (36). Empirically, generation of a CTL response to this particular domain would afford a double benefit to the host, first, by destroying the infected cells and, second, by selecting for nonviable mutants since at least two of these functions are known to be important in the viral life cycle.

Finally, if we can demonstrate that chronically HBV-infected HLA-A31- and Aw68-positive patients respond poorly to HBcAg 141-151, as we have demonstrated for the HLA-A2-restricted response to HBcAg 18-27 (13), it may represent a second potential target for future CTL-based, immunotherapeutic strategies designed to terminate chronic HBV infection. Since such strategies are HLA haplotype dependent, the known world-wide prevalence of the HLA-A2, HLA-A31, and Aw68 alleles (37) suggests that the successful induction of a CTL response to HBcAg 18-27 could benefit ~45% of chronically infected patients, and that another 15-20% could benefit from induction of a therapeutic HBcAg 141-151-specific CTL response.

We thank Drs. Antonella Vitiello and Robert Chesnut (Cytel Corporation, San Diego, CA) for valuable discussions and synthetic peptides. We also thank Dr. Donald Wiley for providing the HLA-Aw68.2-positive cell line and for sharing the results of his structural analysis of the HLA-Aw68.1 binding motif before publication.

This work was supported by National Institutes of Health grants A1-20001 (F. V. Chisari) and RR-00833 to the General Clinical Research Center at Scripps Clinic. This is manuscript number 7527-MEM from The Scripps Research Institute.

Address correspondence to Francis V. Chisari, Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037.

Received for publication 13 October 1992 and in revised form 14 December 1992.

Note added in proof: While this manuscript was under review, Guo et al. and Silver et al. (38, 39) demonstrated, by structural analysis, that the HLA-Aw68.1 molecule binds peptides containing a threonine residue in position 2, a hydrophobic residue (such as leucine) in position 3, and an arginine residue at the carboxy terminus. Since HBcAg 141-151 contains this precise motif, and since we had not identified threonine 142 as an important binding residue for HLA-Aw68, or for HLA-A31, in our alanine substitution studies (Fig. 8), we examined the ability of a peptide containing a nonconservative tryptophan residue at this position (T142W) to prime target cells for recognition by clones 2D7 (HLA-Aw68 restricted) and 3D11 (HLA-A31 restricted). In a peptide dose titration experiment we recently determined that peptide T142W was recognized 100-1,000-fold less efficiently by clones 2D7 and 3D11 in comparison with the native peptide (not shown). These new findings are compatible with the recently published structural data mentioned above (38, 39), and they suggest that threonine in position 2 may also serve as an anchor residue for the binding of immunogenic peptides to HLA-A31 as well.

References

1. Tiollais, P., C. Pourcel, and A. Dejean. 1985. The hepatitis B virus. *Nature (Lond.)* 317:489.
2. Chisari, F.V., C. Ferrari, and M. Mondelli. 1989. Hepatitis B virus structure and biology. *Microb Pathog.* 6:311.
3. Ferrari, C., A. Penna, T. Giuberti, M.J. Tong, E. Ribera, F. Fiaccadori, and F.V. Chisari. 1987. Intrahepatic nucleocapsid antigen-specific T cells in chronic active hepatitis B. *J. Immunol.* 139:2050.
4. Barnaba, V., A. Franco, A. Alberti, C. Balsano, R. Venvenuto, and F. Balsano. 1989. Recognition of hepatitis B virus envelope proteins by liver-infiltrating T lymphocytes in chronic HBV infection. *J. Immunol.* 143:2650.
5. Ferrari, C., M.U. Mondelli, A. Penna, F. Fiaccadori, and F.V. Chisari. 1987. Functional characterization of cloned intrahepatic, hepatitis B virus nucleocapsid-specific helper T cell lines. *J. Immunol.* 139:539.
6. Mondelli, M., G. Mieli-Vergani, A. Alberti, D. Vergani, B. Portmann, A.L.W.F. Eddleston, and R. Williams. 1982. Specificity of T lymphocyte cytotoxicity to autologous hepatocytes in chronic hepatitis virus infection: evidence that cells are directed against HBV core antigen expressed on hepatocytes. *J. Immunol.* 129:2773.
7. Townsend, A.R.M., J. Rothbard, F.M. Gotch, G. Bahadur, D. Wraith, and A.J. McMichael. 1986. The epitopes of Influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. *Cell* 44:959.
8. Nixon, D.F., A.R.M. Townsend, J.G. Elvin, C.R. Rizza, J. Gallwey, and A.J. McMichael. 1988. HIV-1 gag-specific cytotoxic T lymphocytes defined with recombinant vaccinia virus and synthetic peptides. *Nature (Lond.)* 336:484.
9. Yewdell, J.W., J.R. Bennink, M. Mackett, L. Lefrancois, D.S. Lyles, and B. Moss. 1986. Recognition of cloned vesicular stomatitis virus internal and external gene products by cytotoxic T lymphocytes. *J. Exp. Med.* 163:1529.
10. Holt, C.A., K. Osorio, and F. Lilly. 1986. Friend virus-specific cytotoxic T lymphocytes recognized both *gag* and *env* gene-encoded specificities. *J. Exp. Med.* 164:211.
11. Bangham, C.R.M., P.J.M. Openshaw, L.A. Ball, A.M.Q. King, G.W. Wertz, and B.A. Askonas. 1986. Human and murine cytotoxic T cells specific to respiratory syncytial virus recognize the viral nucleoprotein (N), but not the major glycoprotein (G), expressed by vaccinia virus recombinants. *J. Immunol.* 137:3973.
12. Bertolotti, A., F.V. Chisari, A. Penna, G. Missale, A. Cavalli, P. Fowler, and C. Ferrari. 1993. A 10 amino acid peptide defines the optimal sequence recognized by HLA-A2 restricted hepatitis B virus nucleocapsid-specific cytotoxic T cells. *J. Virol.* In press.
13. Penna, A., F.V. Chisari, A. Bertolotti, G. Missale, P. Fowler, T. Giuberti, F. Fiaccadori, and C. Ferrari. 1991. Cytotoxic T lymphocytes recognize an HLA-A2-restricted epitope within the hepatitis B virus nucleocapsid antigen. *J. Exp. Med.* 174:1565.
14. Bertolotti, A., C. Ferrari, F. Fiaccadori, A. Penna, R. Margolske, H.J. Schlicht, P. Fowler, S. Guilhot, and F.V. Chisari. 1991. HLA class I-restricted human cytotoxic T cells recognize endogenously synthesized hepatitis B virus nucleocapsid antigen. *Proc. Natl. Acad. Sci. USA* 88:10445.
15. Pasek, M., T. Goto, W. Gilbert, B. Zink, M. Shaller, P. Mackay, G. Leadbetter, and K. Murray. 1979. Hepatitis B virus genes and their expression in *E. coli*. *Nature (Lond.)* 282:575.
16. Schlicht, H.J., and H. Schaller. 1989. The secretory core protein of human hepatitis B virus is expressed on the cell surface. *J. Virol.* 63:5399.
17. Guilhot, S., P. Fowler, G. Portillo, R.F. Margolske, C. Ferrari, A. Bertolotti, and F.V. Chisari. 1992. Hepatitis B virus (HBV)-specific cytotoxic T-cell response in humans: production of target cells by stable expression of HBV-encoded proteins in immortalized human B-cell lines. *J. Virol.* 66:2670.
18. Tosato, G., 1991. Immunologic studies in humans. In *Current Protocols in Immunology*. Vol 1. Coligan, J.E., A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, and W. Strober, editors. John Wiley & Sons, Inc., New York. 7.22.1-7.22.3.
19. Cerundolo, V., A.G.D. The, R.D. Salter, P. Parham, and A. Townsend. 1991. CD8 independence and specificity of cytotoxic T lymphocytes restricted by HLA-Aw68.1. *Proc. R. Soc. Lond. B Biol. Sci.* 244:169.
20. Eckhardt, S.G., D.R. Milich, and A. McLachlan. 1991. Hepatitis B virus core antigen has two nuclear localization sequences in the arginine-rich carboxyl terminus. *J. Virol.* 65:575.
21. Nassal, M. 1992. The arginine-rich domain of the hepatitis B virus core protein is required for pregenome encapsidation and productive viral positive-strand DNA synthesis but not for virus assembly. *J. Virol.* 66:4107.
22. Sinigaglia, F., M. Guttinger, J. Kilgus, D.M. Doran, H. Matile, H. Etlinger, A. Trzeciak, D. Gillesen, and J.R.L. Pink. 1988. A malaria T-cell epitope recognized in association with most mouse and human MHC class II molecules. *Nature (Lond.)* 336:778.
23. Panina-Bordignon, P., A. Tan, A. Termijtelen, S. Demotz, G. Corradin, and A. Lanzavecchia. 1989. Universally immunogenic T cell epitopes: promiscuous binding to human MHC class II and promiscuous recognition by T cells. *Eur. J. Immunol.* 19:2237.
24. Shirai, M., C.D. Pendleton, and J.A. Berzofsky. 1992. Broad recognition of cytotoxic T cell epitopes from the HIV-1 envelope protein with multiple class I histocompatibility molecules. *J. Immunol.* 148:1657.
25. Johnson, R.P., A. Trocha, T.M. Buchanan, and B.D. Walker. 1992. Identification of overlapping HLA class I-restricted cytotoxic T cell epitopes in a conserved region of the human immunodeficiency virus type 1 envelope glycoprotein: definition of minimum epitopes and analysis of the effects of sequence variation. *J. Exp. Med.* 175:961.
26. Clerici, M., D.R. Lucey, R.A. Zajac, R.N. Boswell, H.M. Gebel, H. Takahashi, J.A. Berzofsky, and G.M. Shearer. 1991. Detection of cytotoxic T lymphocytes specific for synthetic peptides of gp 160 in HIV-seropositive individuals. *J. Immunol.* 146:2214.
27. Bjorkman, P.J., and P. Parham. 1990. Structure, function, and diversity of class I major histocompatibility complex molecules. *Annu. Rev. Biochem.* 59:253.
28. Bjorkman, P.J., M.A. Saper, B. Samraoui, W.S. Bennet, J.L. Strominger, and D.C. Wiley. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature (Lond.)* 329:506.
29. Bjorkman, P.J., M.A. Saper, B. Samraoui, W.S. Bennet, J.L. Strominger, and D.C. Wiley. 1987. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature (Lond.)* 329:512.
30. Garrett, T.P.J., M.A. Saper, P.J. Bjorkman, J.L. Strominger, and D.C. Wiley. 1989. Specificity pockets for the side chains

- of peptide antigens in HLA-Aw68. *Nature (Lond.)* 342:692.
31. Parham, P. 1989. Getting into the groove. *Nature (Lond.)* 342:617.
 32. Falk, K., O. Rotzschke, S. Stevanovic, G. Jung, and H.G. Ramensee. 1991. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature (Lond.)* 351:290.
 33. Hunt, D.F., R.A. Henderson, J. Shabanowitz, K. Sakaguchi, H. Michel, N. Sevilir, A.L. Cox, E. Appella, and V.H. Engelhard. 1992. Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry. *Science (Wash. DC)* 255:1261.
 34. Carman, W.F., M.R. Jacyna, S. Hadziyannis, P. Karayannis, M. McGarvey, A. Makris, and H.C. Thomas. 1989. Mutation preventing formation of e antigen in patients with chronic HBV infection. *Lancet*. ii:588.
 35. Brunetto, M.R., M. Stemmler, F. Schodel, H. Will, A. Ottobrelli, M. Rizzetto, and F. Bonino. 1989. Identification of HBV variants which cannot produce precore-derived HBeAg and may be responsible for severe hepatitis. *Ital. J. Gastroenterol.* 21:151.
 36. Takahashi, K., A. Machida, G. Funatsu, M. Nomura, S. Usuda, S. Aoyagi, K. Tachibana, K. Miyamoto, M. Imai, T. Nakamura, Y. Miyakawa, and M. Mayumi. 1983. Immunochemical structure of hepatitis B e antigen in the serum. *J. Immunol.* 130:2903.
 37. Tiwari, J.L., and P.I. Terasaki. 1985. The HLA complex. In *HLA and Disease Associations*. J.L. Tiwari, and P.I. Terasaki, editors. Springer-Verlag New York, Inc., New York.
 38. Guo, H.-C., T.S. Jardetzky, T.P.J. Garrett, W.S. Lane, J.L. Strominger, and D.C. Wiley. 1992. Different length peptides bind to HLA-Aw68 similarly at their ends but bulge out in the middle. *Nature (Lond.)* 360:364.
 39. Silver, M.L., H.-C. Guo, J.L. Strominger, and D.C. Wiley. 1992. Atomic structure of a human MHC molecule presenting an influenza virus peptide. *Nature (Lond.)* 360:367.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.